International Journal of Applied Pharmaceutics

ISSN- 0975-7058 Vol 15, Issue 1, 2023

Original Article

IDENTIFICATION AND *IN SILICO* ANALYSIS OF INHIBITOR ON THE WNT/β-CATENIN SIGNALING PATHWAY AS POTENTIAL DRUG FOR COLON CANCER

SALBIAH RIDWAN[1], LINDA ERLINA[2], ANTON BAHTIAR[3], DEWI SUKMAWATI[4]

[1,3]Department of Pharmacology, Faculty of Pharmacy, Universitas Indonesia, Gedung Fakultas Farmasi Kampus UI Depok 16424, Indonesia, [2]Department of Medicinal Chemistry, Faculty of Medicine, Universitas Indonesia, Jl. Salemba Raya No. 6, Indonesia, [4]Department of Histology, Faculty of Medicine, Universitas Indonesia, Jl. Salemba Raya No. 6, Indonesia
Email: salbiah@ui.ac.id

Received: 11 Oct 2022, Revised and Accepted: 10 Nov 2022

ABSTRACT

Objective: We aimed to predict the PPI network and *in silico* analysis of a drug that can potentially inhibit colon cancer, specifically in the Wnt/β-catenin signaling pathway, based on pharmacophore modeling and molecular docking.

Methods: Target genes involved in colon development were screened for specific genes in the Wnt/b-catenin signaling pathway. Tissue construction and possible signaling pathways were analyzed using protein-protein interactions. Genes with significant centrality and best-grade values were made to feature pharmacophore models and their suitability for potential drugs. Validation was carried out using the molecular docking method for interaction with the best Hits.

Results: Protein-Protein Interaction Network (PPI) revealed BTNNB1, TP53, AXIN, FZD-8, and CDK1 as potential critical targets in the Wnt/β-catenin signaling pathway and from the suitability of pharmacophore features obtained 27 drugs as the best Hit compounds. The therapeutic effects of the drugs we found were shown to be related to the synergistic activity (multitarget and multi-path). GO enrichment analysis revealed 36 GO entries, including 11 biological processes, 10 cellular components, and 15 molecular functions. Molecular docking experiments confirmed the correlation between three drugs (Clofazimine, Closantel, and Sulindac) with the best binding to 4 target proteins (AXIN1, TP53, CDK1, and FZD-8).

Conclusion: In this study, we found a potent drug that can inhibit colon cancer disease in the Wnt/β-catenin signaling pathway and an essential target protein responsible for the efficacy of colon cancer treatment, providing a theoretical basis for further research.

Keywords: Colon cancer, Wnt/β-catenin signaling pathway, Protein-protein interaction network, Pharmacophore modeling, Molecular docking

DOI: https://dx.doi.org/10.22159/ijap.2023v15i1.46570. Journal homepage: https://innovareacademics.in/journals/index.php/ijap

INTRODUCTION

Colon cancer is an abnormal growth of cells in the tissues of the colon or rectum. Colon cancer is also known as colorectal adenocarcinoma. This cancer usually arises from the glandular epithelial cells of the colon. Colon cancer grows when specific cells in the epithelial tissue undergo a series of genetic or epigenetic mutations that provide opportunities for these cells to develop [1]. These genetic disorders can be caused by lifestyle, personal characteristics, and inheritance [2]. Colon cancer that occurs genetically has hundreds of mutations in different genes, but the number of mutated genes that promote the development of carcinogenesis is still limited.

Based on GLOBOCAN data in 2020, colon cancer ranks fourth in the most cancer cases worldwide, and rectal and colorectal cancer (CRC) ranks third most frequently diagnosed cancer globally, with 10% of all cancers diagnosed globally. Interpreted in the world, as well as being the second leading cause of death from all cancers in 2020, with a total of 935 173 (9.4%) [3, 4]. In Indonesia, colorectal cancer is the second most common type of cancer (male and female), with the addition of 396,914 new cases based on GLOBOCAN data in 2020 [5].

The intestine plays a significant role in the digestion of food in living things, so essentialortant to maintain a balance in the growth of intestinal tissue. The intestinal growth process is regulated by several signaling pathways, including Wnt/β-catenin as the primary driver of intestinal stem cell (ISC) proliferation and maintenance. β-catenin signaling is a signaling pathway that plays an essential role in various regulations in many developmental and biological processes. Several signaling processes influenced by the-catenin pathway are proliferation, differentiation, apoptosis, migration, invasion, and tissue homeostasis [6-8]. The β-catenin-dependent signaling pathway occurs via the binding of the Cysteine-rich glycoprotein ligand to the LRP-5/6 and FZD receptors. The signaling process occurs through the binding of Wnt ligands, and their receptors on the cell surface induce disheveled (DVL), resulting in aggregation of complexes (AXIN, GSK3β, CK1D, and APC). Under pathophysiological conditions, the accumulation of the complexes (AXIN, GSK3β, CK1D, and APC) did not occur, so the concentration of-catenin in the cytoplasm increased. Catenin will migrate to the nucleus and will interact with T-cell specific factor (TCF)/lymphoid enhancer-binding factor (LEF) and coactivators such as pygopus, and Bcl-9, thereby triggering the activation of target genes in the Wnt pathway, namely c-Myc, cyclin D1, and CDKN1A, resulting in upregulation of TCF/lEF target genes and abnormal cell growth [9].

Targeted drugs used in cancer treatment are still minimal. To date, targeted therapies that have been approved for the treatment of colon cancer are limited to the EGFR and VEGF pathways, such as cetuximab, bevacizumab, panitumumab, Ziv-aflibercept, regorafenib, ramucirumab, pembrolizumab, nivolumab, and ipilimumab [10]. One approach to facilitate the discovery of targeted anticancer drugs, drug repurposing, is the right alternative strategy to find new indications of drugs that have been approved.

In this study, we used drugs from the class of NSAIDs, antibiotics, and anti-parasites that have previously been shown to affect cancer treatment, assuming that drugs from the same class have similar activity. Our search results obtained 47 drug candidates, which were then analyzed using protein-protein interactions and compared with a pharmacophore modeling approach (table 2). The consensus candidate compounds obtained from both methods were then validated using molecular docking and molecular dynamics.

MATERIALS AND METHODS

Gene target screening of colon cancer in the wnt/β-catenin signaling pathway

Target gene screening was conducted using the GeneCards Suite website (https://genecards.org) [11]. GeneCards is an integrative database that provides comprehensive human gene information and

integrates centric genes from 125 sources, including genomic, transcriptomics, proteomics, genetic, clinical, and functional [12]. We used the keyword "colon cancer [AND] Wnt canonical signaling [AND] proliferation [AND apoptosis [AND] cell cycle" to search for colon cancer target genes in the Wnt/b-catenin signaling pathway.

Analysis of protein-protein interaction (ppi) and protein-ligand interaction (PLI) network

Protein preparation

genes involved in the mechanism of colon cancer in the Wnt pathway obtained from GeneCard. Before analyzing protein-protein or protein-ligand interactions, it is necessary to prepare protein to get good analytical results. In this study, we used Cytoscape 4.2.8 software to assist in protein preparation [13]. The first step is to separate the coding protein from other impurities. An additional application needed for protein preparation is STRING which is installed through the network in Cytoscape to display the stringed score value (≥0.99).

Protein-protein interaction analysis

Analysis was carried out via the STRING database website (https://string-db.org) to show protein-protein interactions involved in Wnt signaling in colon cancer [14].

Protein-ligand interaction analysis

This study uses the STITCH (https://stitch.embl.de/) databases to analyze the interaction of target proteins and ligands [15].

Structure-based pharmacophore modeling

Drugs that potentially inhibit colon cancer in the Wnt/β-catenin pathway were obtained through the literature. A structure-based pharmacophore model was created, 47 2D drug structures were obtained through PubChem (https://pubchem.ncbi.nlm.nih.gov) [16], and receptors that bind to drug candidates were tested through protein-ligand interactions. The results of the protein-ligand interaction showed that five proteins were attached to the drug candidate (table 1). Five protein structures of the Wnt complex with a human inhibitor (homo sapiens) were obtained through X-Ray Diffraction experiments with selected resolution<2.7 Ă downloaded from the Protein Data Bank (https://www.rcsb.org) with codes 7AFW, 3ZDI, 6MXY, 4TN6, dan 6TFB. LigandScout 4.3 software was used in this study to generate a structure-based pharmacophore model [17].

Molecular docking based on virtual screening

Protein and ligand preparation

In the biology computation process, protein preparation is carried out to change the macromolecular structure into a form that can be detected by computer tools [23]. Before the docking process, a protein crystal structure is needed, obtained from the Protein Data Bank (https://www.rcsb.org). The protein used in this study has been demonstrated through protein-protein interactions and supramolecular interactions with ligands in a pharmacophore model. From these results, three proteins were selected 7AFW, 3ZDI, and 6TFB. The downloaded crystal structure in 3D is prepared by adding hydrogen bonds and removing unnecessary atoms or chains through the BIOVIA Discovery Studio Visualiser Tool v21.1.0 software, which is downloaded at https://discover.3ds.com [24]. The 3D ligand crystal structure was downloaded from PubChem and saved in PDB format.

Molecular docking

The prepared proteins and ligands were then analyzed by molecular docking using PyRx virtual filtering software [25]. This device is used to virtually screen potential drug candidates against colon cancer. PyRx includes Autodock and Autodock Vina with the Lamarckian Genetic Algorithm (LGA) to provide assessments. This study used a PyRx device with Vina's AutoDock tools to view the molecular docking interactions. The test results show the binding affinity value (kcal/mol) and are visualized using BIOVIA Discovery Studio Visualiser Tool v21.1.0.

Table 1: Complex proteins and ligands selected from the PDB database

No	PDB code	Ligand code	Name	Crystal resolution	Affinity	Reference
1	7AFW	R9Q	3-[(2~{R})-4-methyl-5-oxidanylidene-2,3-dihydro-1,4-benzoxazepin-2-yl]benzenecarbonitrile	1.81 Å	Kd: 9.15e+5 (nM) from 1 assay(s)	[18]
2	3ZDI	UGJ	3,6-Diamino-4-(2-chlorophenyl)thieno[2,3-b]pyridine-2,5-dicarbonitrile	2.65 Å	-	[19]
3	6MXY	K6M	N-[3-(tert-butyl amino)propyl]-3-(trifluoromethyl)benzamide	1.62 Å	LC50: 2.20e+4 (nM)	[20]
4	4TN6	PFO	4-{4-(4-fluorophenyl)-1-[1-(1,2-oxazole-3-yl methyl)piperidin-4-yl]-1H-imidazol-5-yl}pyrimidin-2-amine	2.41 Å	-	[21]
5	6TFB	N6W	benzo[b][1]benzazepine-11-carboxamide	1.68 Å	Kd: 1.70e+4 (nM)	[22]

RESULTS AND DISCUSSION

Gene target screening of colon cancer in wnt/β-catenin pathway

In this study, GeneCards were used to search for target genes of the Wnt/β-catenin signaling pathway and obtained as many as 5,545 target proteins. Cytocluster used to classify target proteins, the group with the smallest p-value is 9.195E-8, and 202 nodes are accepted. Drugs that potentially inhibit colon cancer in the Wnt/β-catenin signaling pathway were obtained through a literature study and found as many as 47 drugs (table 2).

Analysis of protein-protein (PPI) and protein-ligand interaction network (PLI)

A total of 202 nodes obtained from the cluster results were networked and analyzed using STRING and Cytoscape 4.2.8 software. As a result, 22 targets (consisting of 22 nodes, 159 edges, 14.5 average node degrees, and 18 expected numbers of edges) were selected based on the stringed score (≧0.99). The protein -protein interactions of the target in the Wnt/β-catenin signaling pathway were made using the STRING database (https://string-db.org), and the PPI enrichment p-value<1.0e-16 showed that a very significant interaction occurred (Image 1). Biological process analysis of interacting proteins showed that CTNNB1, AXIN1, DVL2, GSK3B, WNT3, and FZD7/8 were defined as critical genes in the PPI score.

The drug-target interactions and protein-ligand interaction analysis were determined using STITCH (http://stitch.embl.de). A total of 22 protein nodes and 47 drugs were inputted, and an enrichment p-value of 0<0.05 was obtained, which means it has a good significance level. The test results found that some drugs can interact with the target protein while many other drugs only interact with each other without being able to bind to the target (fig. 2).

Go and pathway enrichment analysis

To further investigate the drug mechanism in treating colon cancer in the Wnt/β-catenin signaling pathway, target genes were analyzed for GO enrichment and KEGG pathway. GO enrichment analysis involves biological processes, cellular components, and molecular functions. Twenty-two potential target genes for colon cancer in the Wnt/β-catenin signaling pathway were analyzed using Enrichr (https://maayanlab.cloud/Enrichr), and the top 10 topics of each GO enrichment are shown in fig. 3-5 and tables 3 and 4.

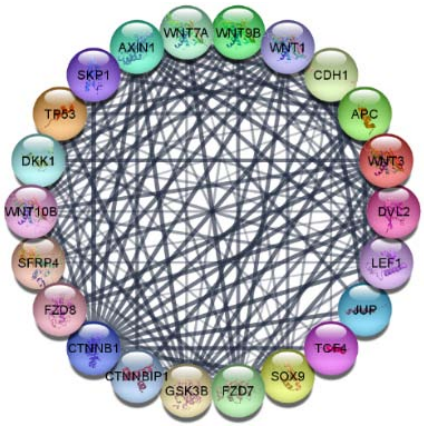


Fig. 1: Colon cancer-related protein network in wnt signaling pathway

Table 2: A list of drugs used for docking against the WNT/β-catenin signaling pathway of colon cancer

Drug name	PubChem ID	Classification	Drug name	PubChem ID	Classification
Acemetacin	CID1981	NSAID	Lornoxicam	CID54690031	NSAID
Albendazole	CID2082	anthelmintic	Mebendazole	CID 4030	anthelmintic
Allopurinol	CID135401907	xanthine oxidase inhibitor	Mefenamic Acid	CID4044	NSAID
Amfenac	CID23663941	NSAID	Meloxicam	CID54677470	NSAID
Amoxicillin	CID33613	penicillin derivative	Mofezolac	CID4237	NSAID
Ampicillin	CID6249	penicillin derivative	Nabumetone	CID 4409	NSAID
Aspirin	CID2244	NSAID	Naproxen	CID156391	NSAID
Cefadroxil	CID47965	cephalosporin antibiotic	Niclosamide	CID4477	anthelmintic
Clofazimine	CID2794	antimycobacterial	Nitazoxanide	CID41684	antimicrobial
Closantel	CID42574	anthelmintic	Oxaprozin	CID 4614	NSAID
Dexketoprofen	CID667550	NSAID	Paracetamol	CID1983	analgesic and antipyretic
Diclofenac	CID3033	NSAID	Piperazine	CID4837	anthelmintic
Emorfazone	CID3221	Analgetic Agent	Piroxicam	CID54676228	NSAID
Epirizole	CID3242	NSAID	Praziquantel	CID4891	anthelmintic
Ethenzamide	CID3282	NSAID	Probenecid	CID4911	uricosuric agent
Etodolac	CID3308	NSAID	Proglumetacin Maleate	CID 5282193	NSAID
Etoricoxib	CID123619	NSAID	Pyrimethamine	CID4993	antiparasitic
Febuxostat	CID134018	xanthine oxidase inhibitor	Pyrvinium Pamoate	CID54680693	anthelmintic
Flubendazole	CID35802	anthelmintic	Salinomycin	CID3085092	antibacterial
Flurbiprofen	CID3394	NSAID	Sulindac	CID1548887	NSAID
Ibuprofen	CID3672	NSAID	Tiaprofenic Acid	CID5468	NSAID
Indomethacin	CID3715	NSAID	Tiaramide Hydrochloride	CID443949	NSAID
Ketoprofen	CID3825	NSAID	Zaltoprofen	CID5720	NSAID
Ketorolac	CID3826	NSAID			

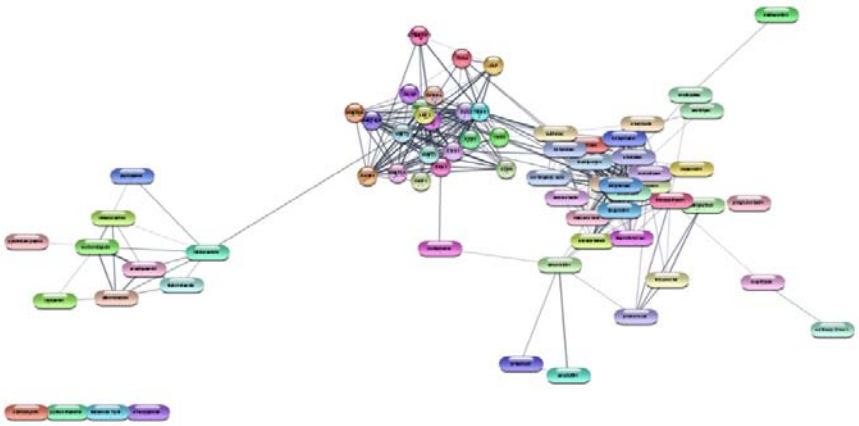


Fig. 2: Protein-protein interaction network of targeted protein and ligand

Fig. 3 shows a bar chart, each representing the top 10 terms from the search results based on the selected gene with the smallest p-value. The color of each bar represents a significant p-value (<0.05)—the sign (*) next to the p-value indicates that the term also has an appropriate significance level. The higher the bar, the smaller the adjusted p-value. The results of GO enrichment in biological processes showed that the Wnt signaling pathway (4.36e-31) had the smallest p-value indicating

the highest significance level. GO enrichment in cellular components showed that the catenin complex (3.3e-08) had the smallest p-value. GO enrichment in molecular function shows that Frizzled binding has the highest significance level with the smallest p-value (3.33e-15).

The top 12 pathways of the KEGG enrichment analysis are shown in fig. 4. The paths consist of the Wnt signaling pathway with the highest significance value (fig. 5A) and the colorectal cancer pathway (fig. 5B) with the most negligible significance in the pathway list from the analysis results.

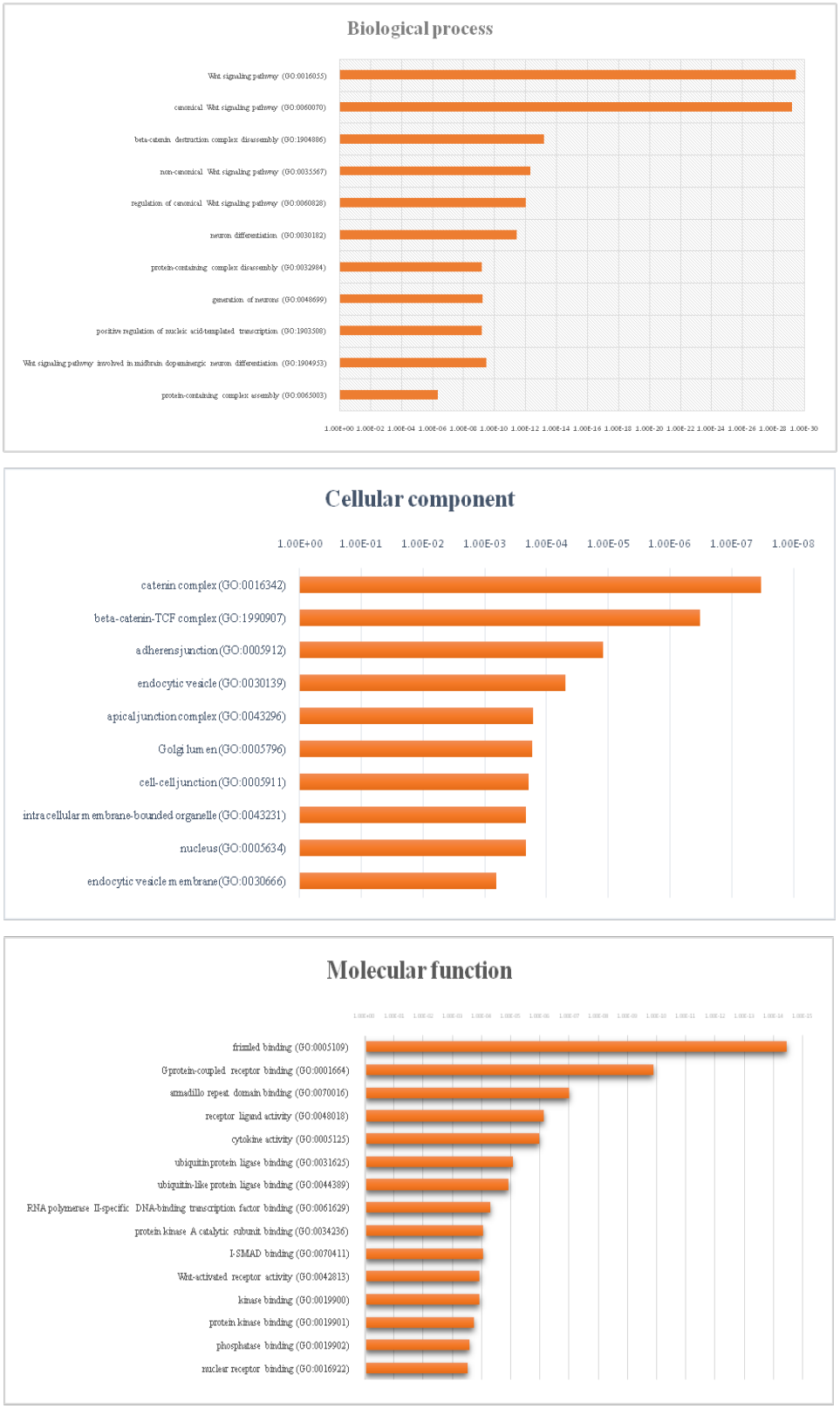


Fig. 3: GO enrichment analysis of biological process, cellular component, and molecular function

Structure-based pharmacophore modeling

This study uses a structure-based pharmacophore model using 3D structural information of the target protein [26]. Five proteins that have an essential role in the development of colon cancer through the Wnt/β-catenin signaling pathway have been previously selected from protein-protein interaction analysis (table 1). Drugs that are active compounds have been once established and can potentially treat colon cancer (table 2).

The pharmacophore model of the five proteins was carried out to determine the unique chemical features that are the site of interaction between the target protein structure with inhibitors or ligands. The analysis results are presented in table 4, which shows the information on the number of chemical features, pharmacophore models, and the number of drugs that hit the compound with the target protein structure. All proteins were selected based on their binding capacity, which had been determined experimentally and validated by the X-ray diffraction method so that the modeling in this study provides optimal and reliable results.

Model-1 shows the structure of the AXIN1 (PDB: 3ZDI) complex with UGJ compound and produces a structure-based pharmacophore model. The results show that three different chemical features, one hydrogen bonding feature and two hydrophobic interaction features, are presented as protein-ligand complex interactions. Some features have been intentionally omitted during pharmacophore modeling to maintain optimal pharmacophore features. The pharmacophore model of model-1 shows that hydrophobic interactions are formed with the amino acid residues of the selected proteins. The hydrogen bonding feature of the donor shown in green is involved in the interaction with several proteins and amino acids ASP200A, while the Sulfur atom in the hydrophobic interaction feature is involved in the interaction with the amino acids ALA83A, LEU132A, LEU188A, and VAL70A. On the other hand, the Cl atom interacts with the ILE62A amino acid and interacts with the N atom and the aromatic ring. Six hit compounds of 47 drugs were found at omitted 0, thus representing that the six compounds had the same three features as in the designed pharmacophore model.

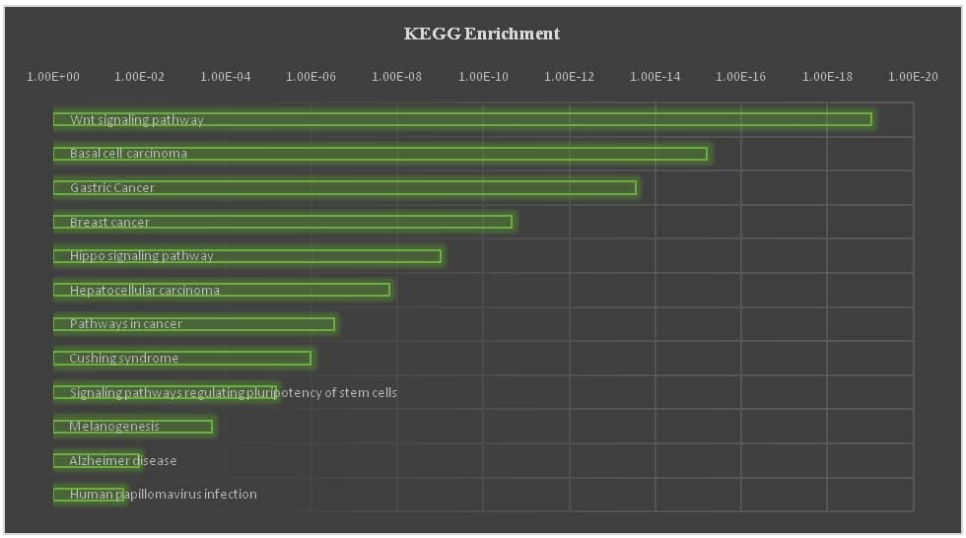


Fig. 4: GO Enrichment of KEGG

Model-2 shows the structure of the protein CDK1 (PDB: 4TN6) complex with the compound PFO401 that forms the pharmacophore model is shown in fig. 5B. The results of the pharmacophore modeling found seven different chemical features, including one hydrogen bonds donor (HBD), two hydrogen bonds acceptor (HBA), one positive ionizable area (PI) feature, and three hydrophobic features (H). In this pharmacophore model, several volume exclusion features that do not interact with amino acids are intentionally omitted to maintain optimal pharmacophore features.

The resulting pharmacophore features of the protein-ligand complex 4TN6 show that the hydrophobic interaction features marked in yellow interact with several selected proteins and amino acids. The hydrophobic feature that binds Fluorine interacts with the amino acids MET80A and ILE68A. The hydrophobic feature that binds to piperidine interacts with the amino acids ALA36A, MET82A, and ILE32A. One other hydrophobic feature also interacts with the amino acids ILE148A and LEU85A, which also interacts with one HBD feature. On the other hand, there are features of PI interacting with amino acid ASP91A and features of HBA interacting with amino acid SER88A. This model found 38 hit compounds from 47 drugs at omitted 4. Each hit compound has three different chemical features.

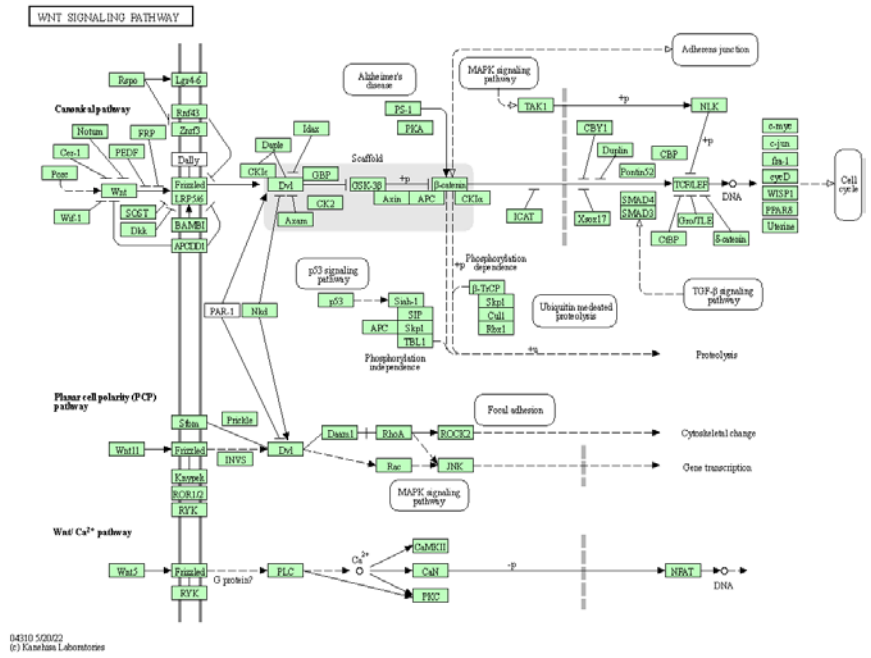
Model-3 displays the structure of the protein TP53 (PDB: 6MXY) complex with K6M compounds selected based on the binding capacity that has been determined experimentally and validated by the X-ray diffraction method. The protein was modeled pharmacophore and produced nine different chemical features. Some features that do not interact with amino acids have been removed to maintain optimal pharmacophore features. The remaining seven features include three green hydrogen bonds donor (HBD), one red hydrogen bonds acceptor (HBA), two yellow hydrophobic (H) features, and one purple positive in the ionizable area (PI).

Three features of HBD bind to NH and interact with the amino acids ASP1521A and ASP1521B. One feature of the HBA interacts with the amino acid ASP1521A, and the feature of PI binds to the NH atom. On the other hand, two hydrophobic features interact with the amino acids MET1584A, MET1584B, and TRY1502A.

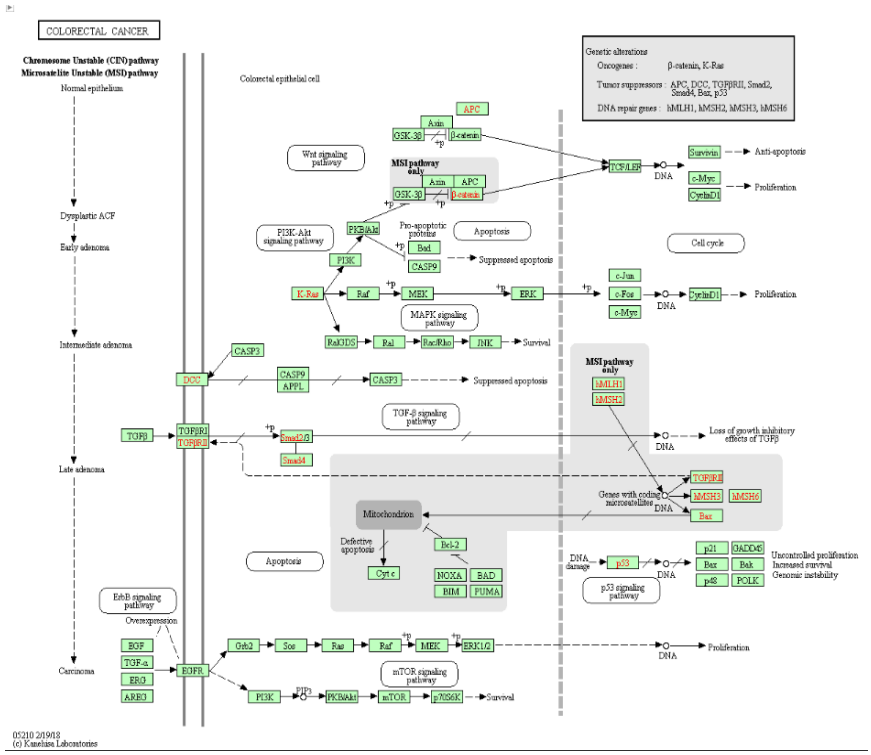
Model-4 displays the structure of the Frizzled-8 protein (GDP: 4TFB), forming a complex with a benzazepine compound. Model 4 yields two of the hydrophobic features of the pharmacophore model. These two features interact with the amino acids LEU138A and MET91A, respectively. Of the 47 drugs screened by model-4, six hit compounds were found at omitted 0, meaning that each hit compound had all the features of the pharmacophore model generated from Frizzled-8.

The last model, model-5, is a protein structure of-catenin (PDB: 7AFW) complex with benzoxazepine compounds and produces three chemical features of the pharmacophore model, including two hydrophobic features and one hydrogen bonds acceptor (HBA) feature. The hydrophobic features interact with the amino acids MET243A and VAL204A, respectively. At the same time, the HBA feature interacts with the amino acid SER246A. A total of 35 hit compounds from 47 drugs were generated from the model at committed 0, meaning that each hit compound had all three features of the 7AFW protein pharmacophore model.

The pharmacophore model of the five proteins in this study was validated using docking with AutodockTools to determine the appropriate target protein and could be analyzed by molecular docking.



(A)



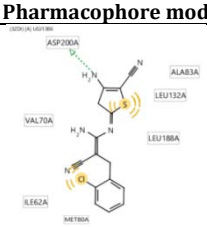
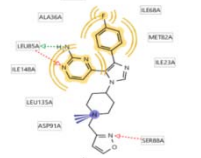
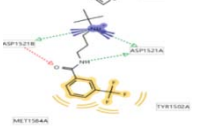
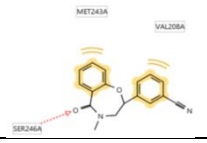
(B)

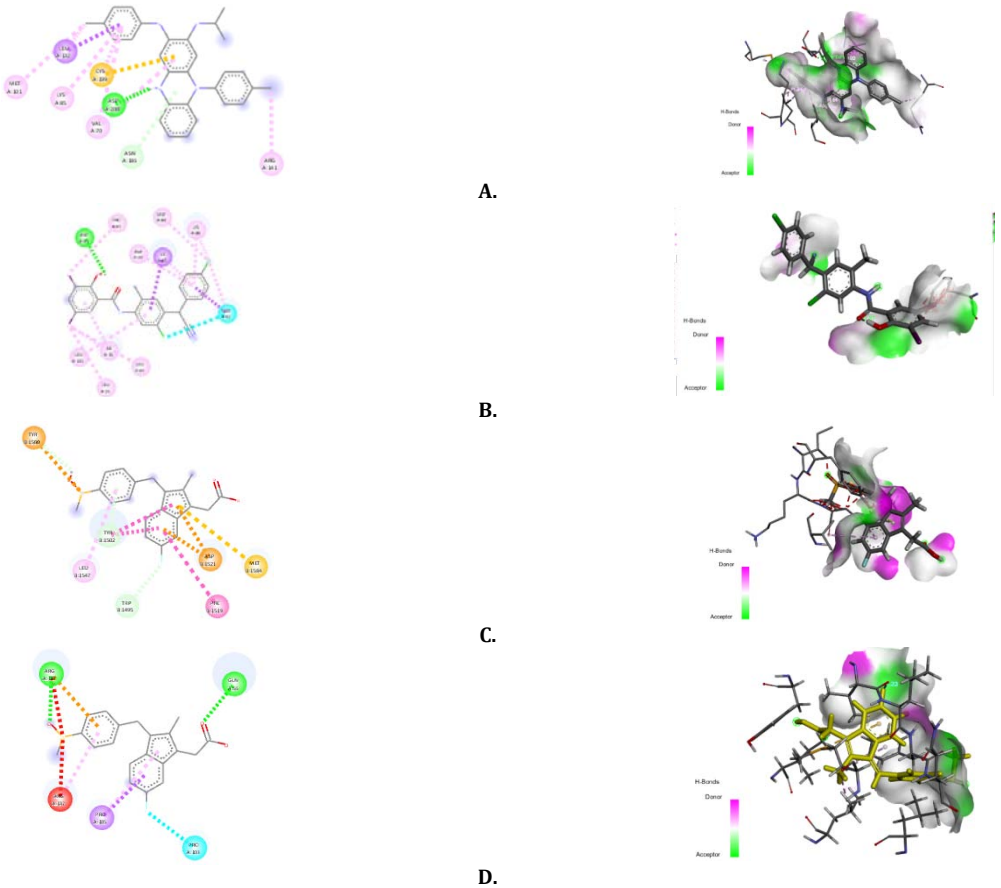
Fig. 5: KEGG pathway, A. Wnt signaling pathway; B. Colorectal cancer pathway

Molecular docking

Twenty-seven drugs (table 2) and five protein target genes (7AFW, 3ZDI, 4TN6, 6MXY, and 4TFB) were selected based on tissue analysis and pharmacophore modeling with high node scores, confidence, and active compound hits. Molecular docking studies in this study were carried out to identify the level of drug interaction and target proteins related to colon cancer genes specific to the Wnt signaling pathway molecularly. In this study, AutodockTools 1.5.6 software was used for structural validation and PyRx for molecular docking. For optimal docking, the docking locations are arranged in a grid at the center of the native ligand.

Table 4: Chemical features of the target protein pharmacophore model and hit compounds

Protein target genes and features	Pharmacophore model	Hit compound
Model-1: 3ZDI Two features hydrophobic One feature HBD		Omitted 0: 6 compound
Model-2: 4TN6 One feature HBD Two features HBA One feature PI Three features Hydrophobic		Omitted 4: 38 compound
Model-3: 6MXY Three features HBD One feature HBA Two features Hydrophobic One feature PI		Omitted 1: 1 compound Omitted 2: 7 compound Omitted 3: 14 compound
Model-3: 6TFB Two features hydrophobic		Omitted 0: 9 compound
7AFW Two features hydrophobic One feature HBA		Omitted 0: 35 compound



A.

B.

C.

D.

Fig. 6: Docking result validation using BIOVIA discovery studio 2D (left) and 3D (right), A. Interaction of amino acid of AXIN1 protein with clofazimine; B. Interaction of amino acid of CKD1 protein with closantel; C. Interaction of amino acid of TP53 with sulindac; D. Interaction of amino acid of FZD-8 receptor with sulindac

A valid molecular docking process can be assessed from the Root Mean Square Deviation (RMSD) parameter of heavy atoms between the molecular docking conformation with experimental results (crystallography) a maximum of 2Å-3Å. The target protein was prepared, and the grid box was set to the center position of the ligands, namely 3ZDI (X =-8,773, Y =-6.277, Z = 8,429), 4TN6 (X =-22,606, Y =-2669, Z = 38255), 6MXY (X =-11,162, Y = 26,776, Z =-4,104), 6TFB (X = 2,711, Y = 12,184, Z =-1.573), and 7AFW (X = 59,556, Y =-40,342, Z = 17.8) with respective dimensions 40 each. Validation results with AutodockTools found four proteins that met the RMSD criteria of less than 3Å, namely 3zdi, 4tn6, 6mxy, and 6tfb. Meanwhile, 7afw protein produces RMSD values more significant than 3Å, so it cannot be used for molecular docking.

After the protein and native ligand validation process, several drugs that were hit compounds were docked again using PyRx Vina to evaluate their binding capacity. Of the 27 drugs, 12 of them produced a higher binding affinity than the native ligand, as shown in table 5. The highest binding affinity for each target protein was clofazimine with 3ZDI (-9.5 kcal/mol), Etodolac with 4TN6 (-9.5 kcal/mol), Sulindac with 6MXY (-8.1 kcal/mol), and Sulindac with 6TFB (-8.0 kcal/mol). Visualization of docking results using BIOVIA Discovery Studio software, as shown in fig. 6.

DISCUSSION

Wnt is a protein that regulates interactions between cells and is one of the pathways for forming colorectal cancer (CRC). Previous studies have demonstrated the potential for targeted treatment of malignant tumors, including colon cancer, but the underlying effector mechanism remains unclear. In this study, we analyzed the potential of drugs to inhibit colon cancer-associated target proteins and found several classes of NSAIDs, anthelmintics, analgesic agents, antimicrobials, and xanthine oxidase inhibitors were highly correlated in tissue, exhibiting significant anti-colon cancer effects [27–29]. For example, aspirin significantly reduced the number and burden of colorectal tumors tested in mice *in vivo* [30]. Niclosamide inhibits CRC growth by targeting the STAT3 pathway [10]. Previous studies have investigated that the Wnt signaling pathway is a signaling pathway that has a high correlation to the development of colon cancer [31]. In this study, we verified that the proteins AXIN1, CDK-1, TP53, and FZD-8 exhibit high binding energies via molecular docking. Downregulation of these four proteins' expression regulation can inhibit colon cancer development [32]. Thus it can be speculated that drugs that can bind to the protein may slow the progression of colon cancer in the Wnt signaling pathway.

Table 5: The docking affinity and interaction of drugs binding to a protein target

Drugs	Binding affinity (Kcal/mol)			
	3zdi/AXIN	4tn6/CDK1	6mxy/TP53	6tfb/FZD-8
Clofazimine	**-9.5**	-9.3	-7.3	-7.1
Flubendazole	-8.4	-9.1	-7.4	-7.1
Sulindac	-8.3	-8.8	-8.1	-8.1
Mebendazole	-8.2	-8.5	-7.4	-7.3
Acetamicin	-8.1	-8.5	-6.6	-7.3
Piroxicam	-7.9	-8.0	-7.6	-6.5
Oxaprozin	-7.9	-8.5	-7.4	-7.2
Etodolac	-7.8	-9.5	-6.5	-7.4
Closantel	-7.8	-8.0	-7.5	-7.1
Mefenamic Acid	-7.5	-8.0	-6.5	-7.1
Amfenac Sodium Hydrate	-7.7	-7.3	-7.2	-6.8
Zaltoprofen	-7.6	-7.5	-7.1	-8.0

3ZDI protein or AXIN1 is a protein that usually forms complexes with other proteins such as APC, GSK3B, and Casein Kinase 1 (CK1) and functions to phosphorylate-catenin. In colon cancer patients, high levels of catenin expression were found [33]. Previous studies have demonstrated that activation of the AXIN/GSK3/APC/CK1 complex can inhibit catenin in the canonical Wnt signaling pathway [34]. 4TN6 is known as Cyclin-Dependent Kinase 1 (CDK1), which is involved in cell cycle regulation [35]. Previous bioinformatics studies have revealed that CDK1 is a candidate target for treating CRC [36]. Increased CDK1 expression has also been observed in tumor cells, including CRC carcinoma, pancreatic ductal adenocarcinoma, and hepatocellular carcinoma [37, 38]. Research has shown that inhibiting CDK1-associated signaling pathways can increase the efficacy of cancer treatment and can reduce 5-Fu resistance [39, 40].

The 6MXY protein, also known as TP53, is a gene that encodes the p53 tumor suppressor protein. Missense mutations in TP53 have been reported to promote the development of colon cancer [41]. TP53 aberrations have been traced to the transition process of the cancer progression sequence from adenoma to carcinoma in colorectal tumorigenesis [42]. The 6TFB protein is FZD-8, encoding the Frizzled-8 receptor involved in the Wnt signaling pathway. Activation of the Frizzled-8 receptor causes Dishevelled activation in the cytosol to initiate the Wnt signaling pathway and liberate catenin from the degradation complex [43]. Inhibition of the FZD8 protein is reported to treat prostate cancer by reducing cell migration, invasion, and cell growth and simultaneously inhibiting Wnt and TGF-β signaling pathways [44]. The conclusion is that targeted drug-drug interactions with target proteins in the Wnt/β-catenin signaling pathway provide significant advantages in developing targeted colon cancer therapeutic drugs.

As a result of the PPI network analysis, CTNNB1 was defined as a critical gene in the Wnt/β-catenin signaling pathway. CTNNB1 is also known as Catenin Beta 1 or the gene encoding β-catenin. CTNNB1 gene mutations are associated with increased expression of the Wnt/β-catenin signaling pathway and decreased quality of life in colon cancer patients [45]. This discovery may be a potential key target in the treatment of colon cancer.

GO enrichment analysis found that gene target proteins are involved in many other activities, such as neuronal differentiation, adherent junctions, and cytokine activity. The results of the KEGG enrichment analysis show that in addition to colon cancer-associated enrichment in the Wnt signaling pathway, target genes are also concentrated in Alzheimer's disease, the planar cell polarity change pathway, and are involved in other signaling pathways such as the MAPK signaling pathway, TP53 signaling pathway, and TGF-β signaling pathway. Relevant studies have shown that activation of these pathways further promotes the development of colon cancer [46]. The MAPK pathway is responsible for cell growth, survival, angiogenesis, and neoplastic cell metastasis [31]. TP53 signaling pathway plays a role in cellular processes such as apoptosis and cancer development linked to the loss of function of p53 [47]. The TGF-β pathway plays a role in cell growth, division, and adhesion, stimulating apoptosis and cell differentiation [31].

Based on the results of our investigation using drugs that the FDA has approved through the PPI network and pharmacophore modeling, 27 drugs and four target proteins were selected for docking. The molecular docking results showed 12 drugs with excellent and stable binding affinity to the four target proteins: AXIN1, CDK1, TP53, and FZD-8.

From a tissue pharmacology perspective, this study initially explored the literature regarding potential anti-colon cancer drugs and cleaves target proteins in the Wnt/β-catenin signaling pathway in CRC, providing a theoretical basis for further experimental

verification. Given the limitations of tissue pharmacology, the pharmacological mechanism of drugs analyzed in treating colon cancer according to the target on the Wnt/β-catenin pathway is only predicted through data mining. The interactions between drugs and proteins need to be verified further through pharmacological and clinical studies.

CONCLUSION

In summary, this study demonstrates the effector mechanisms of previously FDA-approved drugs with different indications and potential in treating colon cancer with specific targets in the Wnt/β-catenin signaling pathway based on PPI analysis and *in silico* studies. We reveal that Clofazimine, Flubendazole, and Sulindac are essential in inhibiting the target proteins in the Wnt pathway, namely FZD-8, AXIN1, TP53, and CDK1. The molecular docking results also show that the three drugs can combine well with the target protein and provide an essential basis for further research. However, this study also has certain pharmacological and clinical limitations, so validating our findings is still needed.

FUNDING

Nil

AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

REFERENCES

1. Chen W, Zheng R, Baade PD, Zhang S, Zeng H, Bray F. Cancer statistics in China, 2015. CA Cancer J Clin. 2016;66(2):115-32. doi: 10.3322/caac.21338, PMID 26808342.
2. Church J. Molecular genetics of colorectal cancer. Semin Colon Rect Surg. 2016;27(4):172-5. doi: 10.1053/j.scrs.2016.04.013.
3. Dekker E, Tanis PJ, Vleugels JLA, Kasi PM, Wallace MB. Colorectal cancer. Lancet. 2019;394(10207):1467-80, doi: 10.1016/S0140-6736(19)32319-0.
4. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2021;71(3):209-49. doi: 10.3322/caac.21660, PMID 33538338.
5. Pangribowo S, Kanker B. di Indonesia. Pus Data Dan Inf Kesehat Kementeri Kesehat RI; 2019. p. 1-16.
6. Koncina E, Haan S, Rauh S, Letellier E. Prognostic and predictive molecular biomarkers for colorectal cancer: updates and challenges. Cancers (Basel). 2020;12(2):1-25. doi: 10.3390/cancers12020319, PMID 32019056.
7. Salik B, Yi H, Hassan N, Santiappillai N, Vick B, Connerty P. Targeting RSPO3-LGR4 signaling for leukemia stem cell eradication in acute myeloid leukemia. Cancer Cell. 2020;38(2):263-278.e6. doi: 10.1016/j.ccell.2020.05.014, PMID 32559496.
8. Soleas JP, D'Arcangelo E, Huang L, Karoubi G, Nostro MC, McGuigan AP. Assembly of lung progenitors into developmentally inspired geometry drives differentiation via cellular tension. Biomaterials. 2020;254(May):120128. doi: 10.1016/j.biomaterials.2020.120128. PMID 32474250.
9. Zhang Y, Wang X. Targeting the Wnt/β-catenin signaling pathway in cancer. J Hematol Oncol. 2020;13(1):165. doi: 10.1186/s13045-020-00990-3, PMID 33276800.
10. Xie YH, Chen YX, Fang JY. Comprehensive review of targeted therapy for colorectal cancer. Signal Transduct Target Ther. 2020;5(1):22. doi: 10.1038/s41392-020-0116-z, PMID 32296018.
11. Safran M, Rosen N, Twik M, BarShir R, Stein TI, Dahary D. The gene cards suite. Pract Guide to Life Sci Databases; 2021. p. 27-56.
12. Stelzer G, Rosen N, Plaschkes I, Zimmerman S, Twik M, Fishilevich S. The GeneCards suite: from gene data mining to disease genome sequence analyses. Curr Protoc Bioinformatics. 2016;54:1.30.1-1.30.33. doi: 10.1002/cpbi.5, PMID 27322403.
13. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D. Cytoscape: A software environment for integrated models of biomolecular interaction networks. Genome Res. 2003;13(11):2498-504. doi: 10.1101/gr.1239303, PMID 14597658.
14. Jensen LJ, Kuhn M, Stark M, Chaffron S, Creevey C, Muller J. STRING 8-A global view on proteins and their functional interactions in 630 organisms. Nucleic Acids Res. 2009;37Suppl 1:D412-6. doi: 10.1093/nar/gkn760, PMID 18940858.
15. Kuhn M, von Mering C, Campillos M, Jensen LJ, Bork P. STITCH: interaction networks of chemicals and proteins. Nucleic Acids Res. 2008;36Suppl 1:D684-8. doi: 10.1093/nar/gkm795, PMID 18084021.
16. Kim S, Chen J, Cheng T, Gindulyte A, He J, He S. PubChem in 2021: new data content and improved web interfaces. Nucleic Acids Res. 2021;49(D1):D1388-95. doi: 10.1093/nar/gkaa971, PMID 33151290.
17. Wolber G, Langer T. LigandScout: 3-D pharmacophores derived from protein-bound ligands and their use as virtual screening filters. J Chem Inf Model. 2005;45(1):160-9. doi: 10.1021/ci049885e, PMID 15667141.
18. Kessler D, Mayer M, Zahn SK, Zeeb M, Wohrle S, Bergner A. Getting a grip on the undrugged: targeting β-catenin with fragment-based methods. ChemMedChem. 2021;16(9):1420-4. doi: 10.1002/cmdc.202000839, PMID 33275320.
19. Fugel W, Oberholzer AE, Gschloessl B, Dzikowski R, Pressburger N, Preu L, *et al.* Fugel 2013HYDE. J Med Chem. 2012;56(1).
20. Dai Y, Zhang A, Shan S, Gong Z, Zhou Z. Structural basis for recognition of 53BP1 tandem Tudor domain by TIRR. Nat Commun. 2018;9(1):2123. doi: 10.1038/s41467-018-04557-2, PMID 29844495.
21. Madej T, Lanczycki CJ, Zhang D, Thiessen PA, Geer RC, Marchler Bauer A. MMDB and VAST+: tracking structural similarities between macromolecular complexes. Nucleic Acids Res. 2014;42:D297-303. doi: 10.1093/nar/gkt1208. PMID 24319143.
22. Zhao Y, Ren J, Hillier J, Lu W, Jones EY. Antiepileptic drug carbamazepine binds to a novel pocket on the Wnt receptor Frizzled-8. J Med Chem. 2020;63(6):3252-60. doi: 10.1021/acs.jmedchem.9b02020, PMID 32049522.
23. Scott DE, Ehebauer MT, Pukala T, Marsh M, Blundell TL, Venkitaraman AR. Using a fragment-based approach to target protein-protein interactions. ChemBioChem. 2013;14(3):332-42. doi: 10.1002/cbic.201200521, PMID 23344974.
24. Dassault Systemes BIOVIA. Discovery studio modeling environment. Release 2021. Dassault Systemes: San Diego. CA; 2021.
25. Dallakyan S, Olson AJ. Small-molecule library screening by docking with PyRx. Methods Mol Biol. 2015;1263:243-50. doi: 10.1007/978-1-4939-2269-7_19, PMID 25618350.
26. Chen C, Wang T, Wu F, Huang W, He G, Ouyang L. Combining structure-based pharmacophore modeling, virtual screening, and *in silico* ADMET analysis to discover novel tetrahydro-quinoline based pyruvate kinase isozyme M2 activators with antitumor activity. Drug Des Devel Ther. 2014;8:1195-210. doi: 10.2147/DDDT.S62921, PMID 25214764.
27. Zappavigna S, Cossu AM, Grimaldi A, Bocchetti M, Ferraro GA, Nicoletti GF. Anti-inflammatory drugs as anticancer agents. Int J Mol Sci. 2020;21(7):1-29. doi: 10.3390/ijms21072605, PMID 32283655.
28. Durusu İZ, Husnugil HH, Atas H, Biber A, Gerekci S, Gulec EA. Anti-cancer effect of clofazimine as a single agent and in combination with cisplatin on U266 multiple myeloma cell line. Leuk Res. 2017;55:33-40. doi: 10.1016/j.leukres.2017.01.019, PMID 28122281.
29. Nowak Sliwinska P, Scapozza L, Ruiz I Altaba A. Drug repurposing in oncology: Compounds, pathways, phenotypes and computational approaches for colorectal cancer. Biochim Biophys Acta Rev Cancer. 2019;1871(2):434-54. doi: 10.1016/j.bbcan.2019.04.005. PMID 31034926.
30. Zhao R, Coker OO, Wu J, Zhou Y, Zhao L, Nakatsu G. Aspirin reduces colorectal tumor development in mice and gut microbes reduce its bioavailability and chemopreventive effects. Gastroenterology. 2020;159(3):969-83.e4. doi: 10.1053/j.gastro.2020.05.004. PMID 32387495.

31. Koveitypour Z, Panahi F, Vakilian M, Peymani M, Seyed Forootan F, Nasr Esfahani MH. Signaling pathways involved in colorectal cancer progression. Cell Biosci. 2019;9(1):97. doi: 10.1186/s13578-019-0361-4, PMID 31827763.
32. Zhang X, Wang L, Qu Y. Targeting the β-catenin signaling for cancer therapy. Pharmacol Res. 2020;160:104794. doi: 10.1016/j.phrs.2020.104794, PMID 32278038.
33. Nazemalhosseini Mojarad E, Kashfi SMH, Mirtalebi H, Almasi S, Chaleshi V, Kishani Farahani R. Prognostic significance of nuclear β-catenin expression in patients with colorectal cancer from Iran. Iran Red Crescent Med J. 2015;17(7):e22324. doi: 10.5812/ircmj.22324v2, PMID 26421170.
34. Zhao H, Ming T, Tang S, Ren S, Yang H, Liu M. Wnt signaling in colorectal cancer: pathogenic role and therapeutic target. Mol Cancer. 2022;21(1):144. doi: 10.1186/s12943-022-01616-7, PMID 35836256.
35. Malumbres M. Cyclin-dependent kinases. Genome Biol. 2014;15(6):122. doi: 10.1186/gb4184, PMID 25180339.
36. Li J, Wang Y, Wang X, Yang Q. CDK1 and CDC20 overexpression in patients with colorectal cancer are associated with poor prognosis: evidence from integrated bioinformatics analysis. World J Surg Oncol. 2020;18(1):50. doi: 10.1186/s12957-020-01817-8. PMID 32127012.
37. Dong S, Huang F, Zhang H, Chen Q. Tumor tissues predicts poor survival in pancreatic ductal adenocarcinoma. Gene 2019;15:1-10.
38. Wu CX, Wang XQ, Chok SH, Man K, Tsang SHY, Chan ACY. Blocking CDK1/PDK1/β-catenin signaling by CDK1 inhibitor RO3306 increased the efficacy of sorafenib treatment by targeting cancer stem cells in a preclinical model of hepatocellular carcinoma. Theranostics. 2018;8(14):3737-50. doi: 10.7150/thno.25487, PMID 30083256.
39. Huang J, Chen P, Liu K, Liu J, Zhou B, Wu R. CDK1/2/5 inhibition overcomes IFNG-mediated adaptive immune resistance in pancreatic cancer. Gut. 2021;70(5):890-9. doi: 10.1136/gutjnl-2019-320441, PMID 32816920.
40. Zhu Y, Li K, Zhang J, Wang L, Sheng L, Yan L. Inhibition of cdk1 reverses the resistance of 5-fu in colorectal cancer. Cancer Manag Res. 2020;12:11271-83. doi: 10.2147/CMAR.S255895, PMID 33177877.
41. Williams DS, Mouradov D, Browne C, Palmieri M, Elliott MJ, Nightingale R. Overexpression of TP53 protein is associated with the lack of adjuvant chemotherapy benefit in patients with stage III colorectal cancer. Mod Pathol. 2020;33(3):483-95. doi: 10.1038/s41379-019-0353-2, PMID 31471586.
42. Fearon RE, Vogelstein B. A genetic model for colorectal tumorgenesis. G Ital Cardiol. 1990;19(2):170-2.
43. Alrefaei AF. Frizzled receptors (FZD) play multiple cellular roles in development, in diseases, and as potential therapeutic targets. Journal of King Saud University–Science. 2021;33:101613. doi: 10.1016/j.jksus.2021.101613.
44. Murillo Garzon V, Gorrono Etxebarria I, Akerfelt M, Puustinen MC, Sistonen L, Nees M. Frizzled-8 integrates Wnt-11 and transforming growth factor-β signaling in prostate cancer. Nat Commun. 2018;9(1):1747. doi: 10.1038/s41467-018-04042-w, PMID 29717114.
45. Kurnit KC, Kim GN, Fellman BM, Urbauer DL, Mills GB, Zhang W. CTNNB1 (beta-catenin) mutation identifies low grade, early stage endometrial cancer patients at increased risk of recurrence. Mod Pathol. 2017;30(7):1032-41. doi: 10.1038/modpathol.2017.15, PMID 28281553.
46. Santos VS, Lago NM, Garcia CP, Garcia AP, Apricio LMA. Signaling pathways in CRC. In: Sierra AP, editor. Foundations of colorectal cancer. 1st ed. Academic Press Inc; 2022. p. 519-28.
47. Farooqi AA, de la Roche M, Djamgoz MBA, Siddik ZH. Overview of the oncogenic signaling pathways in colorectal cancer: mechanistic insights. Semin Cancer Biol. 2019;58:65-79. doi: 10.1016/j.semcancer.2019.01.001. PMID 30633978.