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Irinotecan's molecular mechanisms against cancer: a primary system biology and chemoinformatics approach for novel formulation development

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ABSTRACT

Cancer is the third most common type of cancer generally. It affects 6.1% of the entire world's population and kills 9.2% of all people of both sexes. Even though people with colon cancer have a number of chemotherapies and surgeries to choose from, the disease often returns after the first treatment.

AutoDockVina by PyRx 0.8v was used to do molecular docking. The admetSAR2.0 web server was employed for ADMET analysis. The MolSoft and ADVERPred tools were applied to predict the drug's potential for abuse and its potential for side effects.

The anti-tumor effects of irinotecan may be aimed at the metabolic processes and Ras and PI3K-Akt signaling pathways that help cancer grow.

Gene set enrichment and network analysis proved useful in determining possible protein targets of Irinotecan. Molecular docking revealed how Irinotecan and Vitamin E TPGS interact with EGFR. Moreover, we found that vitamin E TPGS possesses the potential to be an efficient inhibitor for the efflux pump substrate medications such as irinotecan. In addition, the network we created was able to demonstrate how pathways contribute to the protein molecules of irinotecan being able to target cancers. Lastly, we conclude that irinotecan's effectiveness in combating colon cancer is due to the formation of a network of protein-pathway links.

INTRODUCTION

Cancer is the largest cause of mortality in the globe, accounting for over 10 million deaths in 2020, or roughly one in every six. Breast, lung, colon, rectal and prostate cancers are the most prevalent. Tobacco use, a high BMI, alcohol consumption, a lack of fruits and vegetables, and a lack of physical activity account for almost one-third of cancer fatalities (https://www.who.int/news-room/fact-sheets/detail/cancer). Infections that cause cancer, such as human papillomavirus and hepatitis, account for around 30% of all cancer cases in low- and lower-middle-income nations. Many tumors can be cured if they are diagnosed early and treated properly [1,2]. Although there are a number of chemotherapies and surgical options for those with colon and breast cancer, the disease often returns after first treatment. Therefore, research into a promising long-term therapeutic therapy for colon cancer is crucial. Phytoconstituents found in herbs have been employed extensively in the treatment of cancers such colon cancer [3-5].

P-gp, or multidrug resistance protein 1, is a permeability glycoprotein. P-gp is a key membrane transporter that plays a crucial role in the metabolism of foreign particles and the subsequent efflux of these particles out of the cell. Adenosine triphosphate (ATP) is required for the substrate-dependent efflux action of P-gp. This protein has been found in a wide variety of microorganisms, including fungi, mammals and bacteria, and is thought to play a role in the defense process against invading organisms or chemicals. P-gp is broadly distributed and plays an important role in the efflux processes of the gut, bile ducts and liver cells, kidney cells (such as proximal tubules) and capillary endothelium – essentially all endothelial cells, including the blood-testis barrier and blood-brain barrier (BBB). It has been found not only in the colon, but also in the pancreas, the adrenal gland, and other organs.

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This protein is frequently over expressed in cancer cells, preventing the entry of many anticancer drugs and limiting the effectiveness of cancer treatment. It protects tissues from potentially harmful noxious elements and aids in the excretion of metabolites, but it is secreted in the digestive tract lumen by the bile ducts. P-gp expression is extensively observed in the majority of cases of cancer, regardless of the kind. Breast cancer chemotherapy often fails because of drug resistance. The epithelial lining of the ducts (85%) or lobules (15%) in the breast glands is the site of cancer development in the majority of cases of breast cancer. When cancer first develops, it is localized (or "in situ") within a duct or lobule and rarely causes symptoms or spreads to other parts of the body. These in situ tumors have the potential to metastasize over time, invading neighboring breast tissue and eventually spreading to regional lymph nodes and beyond. A woman's death from breast cancer is always the result of systemic spread. Multiple laboratories used immunohistochemistry to examine protein expression. In contrast to the main operable breast tumors reported in the several investigations, the protein expression levels of Pgp, Ki-67, and p53 were much greater in the locally progressed breast cancers [6-8].

Camptothecin is found in Camptotheca acuminata, a plant that is native to China and Tibet, and irinotecan is a semi-synthetic derivative of camptothecin that is water soluble. This compound is a highly effective topoisomerase I inhibitor. DNA replication requires topoisomerase I, which covalently attaches to the 30'-phosphorylated end of a nicked DNA strand it generates. Irinotecan is considered to work by stabilising the DNA-topoisomerase I complex, therefore preventing ligation of the nicked DNA strand. The apoptotic cascade is triggered when a replication fork collides with a DNA-topoisomerase I complex that has been stabilized [9-11]. This results in an irreparable DNA double-strand break. Irinotecan undergoes enzymatic breakage of a side chain via systemic carboxylases in vivo, yielding the active metabolite SN-38. This process, mediated by the hepatic uridine diphosphate glucuronosyltransferase 1A1 enzyme, converts SN-38 to the pharmacologically inactive SN-38 glucuronide. SN-38G is eliminated through the kidneys, while its parent molecule is eliminated through the biliary system [11-14].

This study used a network-based systematic approach to investigate the underlying mechanism through which Irinotecan works to treat colorectal cancer. In a nutshell, the drug-target network was built with Irinotecan and colorectal cancer as the disease targets of interest. Through a combination of KEGG pathway enrichment analysis and comparison analysis, we looked at the genes shared by Irinotecan and colorectal cancer. Consequently, the purpose of this investigation was to identify the Irinotecan protein targets that are most likely involved in colon cancer and to record the processes by which they function.

MATERIALS AND METHOD

ADMET profile and drug-like characteristics

The RNN model's drug-likeness score is the sum of the log values of its conditional probability outputs. Predicting ADR from structural information often entails two distinct phases. Firstly, a suitable feature vector is constructed to represent each drug molecule based on its chemical structure. Secondly, an ADR prediction machine learning algorithm is run on the generated feature space (in our study, ADMET profiling was done by using the online software admetSAR 2.0).

The Molecular weight (MW), Lipid/water partition coefficient (log P), Total number of hydrogen bond donor and Total number of hydrogen bond acceptor (NHBA) of the drug Irinotecan were retrieved from online servers, namely, ADVERPred (http://www.way2drug.com/adverpred/) and Swiss ADME (http://www.swissadme.ch/index.php). These are the characteristics that define the drug likeness [20,21].

Target identification

Irinotecan's official SMILE was acquired from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) in addition to its targets being anticipated by the Internet SwissTargetPrediction server (http://www.swisstargetprediction.ch/). Through the use of 2D and 3D configurations with known ligands, the server is able to anticipate molecular protein targets. As an added bonus, the Comparative Toxicogenomics Database was explored to locate the protein molecules that play a role in carcinogenesis (http://ctdbase.org) [11].

Gene set enrichment and network analysis

The STRING database (https://string-db.org/) was fed a list of gene identifiers for proteins thought to be Irinotecan's targets in order to learn more about their relationships. The KEGG pathway was utilised to determine the biological pathways that were regulated by a given collection of genes. Using the assistance of the programme Cytoscape 3.6.1, a network of likely protein targets and Irinotecan's pathways was built. The number of links in the network served as the basis for the study [12].

Docking studies

After the network construction and analysis, the protein targets were selected based on the highest edge counts and larger node size. We selected the seven most potential targets, notably, PIK3CA, ATK1, ATK2, ATK3, EGFR, ERBB and MTOR, the 3D structure of the targets were subsequently retrieved from the structural bioinformatics Protein Data Bank (https://www.rcsb.org/). Furthermore, Auto-dock and Discovery studio visualizers were utilized. We had also docked the vitamin E TPGS with the aforementioned target protein [13].

Preparation of ligands

PubChem database (https://pubchem.ncbi.nlm.nih.gov/) was utilised so as to obtain the three – dimensional structure of irinotecan which was conditioned through Discovery studio [14].

Docking validation

Docking conformation and preparatory processes were completed in the grid box. Discovery studio visualizer was then applied to retrieve the protein structures from

the Protein Data Bank (a repository for structural bioinformatics in the.pdb format (DSV v2019)). Autodock's ligand and target were subsequently transformed into pdbqt format for easier analysis. The defaults for the grid boxes were then selected. Afterwards, using the DSV v2019 programme and the Dock RMSD web server, we evaluated the root mean square of intermolecular interactions and post-docking ligand orientations obtained from PubChem [15].

Molecular docking

The Autodock programme was able to assess the degree of affinity that existed between the target proteins and the medication irinotecan. For the sake of analysis in the autodock, pdbqt files were employed. Via Discover studio visualizer, the results of the analysis received from the autodock were visualized, and the interactions are obtained in both 2D and 3D formats [16].

RESULTS

Target prediction

The predictions made by means of utilizing the Swiss target database led to the discovery of a total of one hundred different targets for the medication irinotecan. We discovered that some targets, specifically PIK3AC, ATK1, ATK2, ATK3, EGRF, ERBB and MTOR, had the ability to counter breast cancer (Table 1). The side effects of the specific drugs are listed in Table 2.

Table 1. Drug likeness characteristics of Irinotecan, Tamoxifen and Vitamin E TPGS

Compound	PubChem CID	Molecular formula	Molecular weight (g/mol)	HBA	HBD	Log P	DLS
Irinotecan	74990	C33H38N4O6	586.28 (>500)	8	1	3.96	1.54
Tamoxifen	2733526	C26H29NO	371.22	2	0	6.20 (>5)	1.49
Vitamin E TPGS		(C2H4O) nC33H54O5	574.42 (>500)	6	1	9.22 (>5)	0.98

Table 2. Probable side effects of Irinotecan, Tamoxifen and Vitamin E TPGS

Compound	Pa	Pi	Side effect
Irinotecan	0.569	0.031	Nephrotoxicity
Tamoxifen	0.824	0.01	Arrhythmia
Tamoxifen	0.708	0.097	Hepatotoxicity
Vitamin E TPGS	No side effects listed		

Analysis of gene-set enrichment and networks

Targets that play a significant part in a variety of pathways were discovered by gene set enrichment analysis. The many cancer-related pathways were extracted from the KEGG pathways, and then, based on the results of a literature review, 18 pathways were chosen that were connected to the various cancer-related pathways. In the network figure, the regulated pathways, as well as the protein targets that were modified, are indicated. The network is built with the degree and edge count as the guiding principles. Figure 1 depicts a network of Irinotecan (purple), probable protein targets, and cancer pathways (blue).

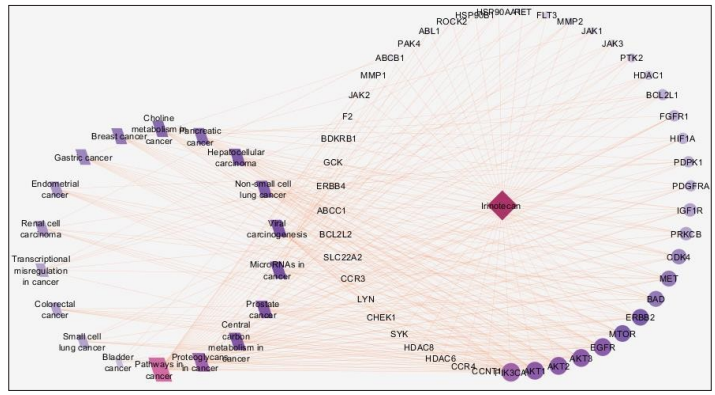

Figure 1. Irinotecan (purple), putative protein targets, and cancer pathways (blue) network

Docking studies

The pharmaceutical agent, irinotecan, was employed in the docking process, which involved seven possible targets. Irinotecan was discovered to have a binding energy of -7.51, which was the lowest of any medication tested with PIK3CA (Figure 2 and Figure 3) (Table 3).

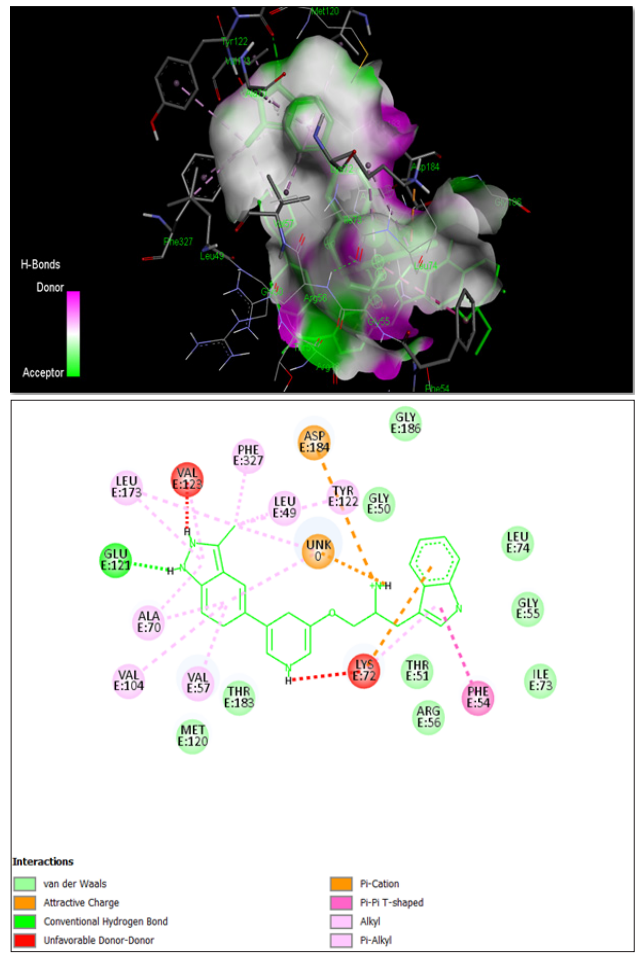

Figure 2. Docking Validation and interaction of Irinotecan and permeation glycoprotein

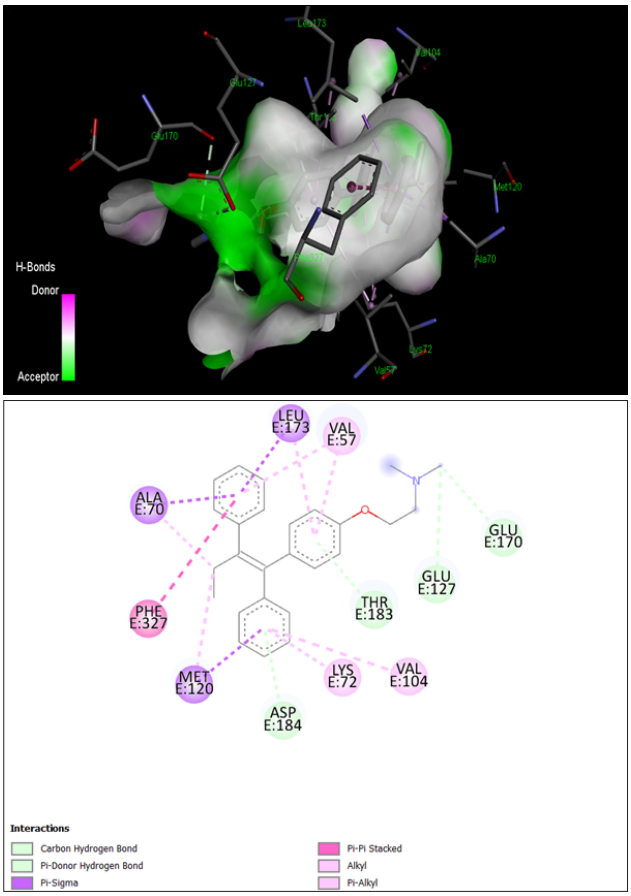

Figure 3. Docking Validation and interaction of Tamoxifen and permeation glycoprotein

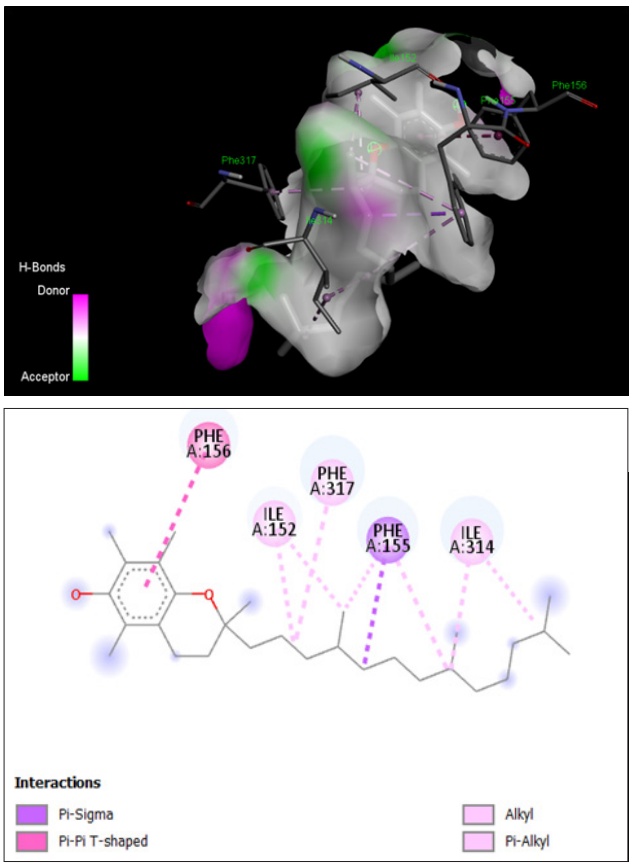

Figure 4. Docking Validation and interaction of Vitamin E TPGS and permeation glycoprotein

Table 3. Results of docking for the glycoprotein and vitamin E

Ligand	Binding Affinity	rmsd/ub	rmsd/lb
P_glycoprotein_SM_vitE	-7.1	0	0
P_glycoprotein_SM_vitE	-7	3.413	1.353
P_glycoprotein_SM_vitE	-7	2.838	1.468
P_glycoprotein_SM_vitE	-6.9	72.746	69.576
P_glycoprotein_SM_vitE	-6.7	3.846	2.543
P_glycoprotein_SM_vitE	-6.5	2.874	1.795
P_glycoprotein_SM_vitE	-6.4	53.18	48.456
P_glycoprotein_SM_vitE	-6.4	6.434	4.084
P_glycoprotein_SM_vitE	-6.3	52.725	49.155

ADMET and drug likeness

Irinotecan had a molecular weight of 586.68 g/mol, which must be less than 500 g/mol. HBA- 8 (10), HBD-1 (5), and LogP-3.73 (5) are the values here. Irinotecan complied with the Lipinski criterion with the exception of one MW value that was more than 500 g/mol. Irinotecan has only been shown to cause Nephrotoxicity, having a Pa value of 0.569 when it comes to potential adverse effects. The outcomes are presented in Table 4.

DISCUSSION

In this study, gene set enrichment, network pharmacology and in silico molecular docking analysis were employed to find the molecular mechanism that is responsible for the anticancer action of irinotecan. These techniques were utilized in order to ascertain the molecular mechanism that is responsible for the anticancer action of irinotecan. This was done with the end goal of understanding how irinotecan stops the multiplication of cancer cells (the primary focus of our efforts). A "network" is an original and innovative approach of anticipating the link between an active pharmacological formulation and protein targets. This method ("network forecasting") is applied in the field of network pharmacology [16-18]. A further objective of our work was to ascertain the binding affinity of the vitamin E TPGS towards the permeation glycoprotein. This investigation was carried out in parallel with the first. According to the findings, there was a considerable degree of interaction and binding score [19]. We are able to reach the conclusion on the basis of the previously reported results, as well as the findings of the current investigation, that vitamin E TPGS possesses the potential to be an efficient inhibitor for the efflux pump substrate medications. The medication irinotecan is just one example of the many different kinds of drugs that are included in this category [20-22].

So as to begin making predictions about the irinotecan targets, we first applied the concept that "similar drugs target similar proteins" in order to determine how to proceed. This was accomplished with the assistance of Swiss Target Prediction and the knowledge that is contained in the Therapeutic Target database concerning protein molecules that are involved in the process of targeting colon cancer [23,24]. Following that, we carried out an enrichment analysis of the compound gene set in order to establish the metabolic

Table 4. Supplementary data on ADMET

ADMET profile	Irinotecan	Tamoxifen	Vitamin E TPGS
Human Intestinal Absorption	0.9766	0.9876	0.9674
Caco-2	0.8305	0.749	0.7445
Blood Brain Barrier	0.7	0.825	0.6
Human oral bioavailability	0.9286	0.8	0.6286
Subcellular localzation	0.4527	0.492	0.8409
OATP2B1 inhibitior	1	1	0.5694
OATP1B1 inhibitior	0.8689	0.9345	0.9094
OATP1B3 inhibitior	0.9209	0.9478	0.8892
MATE1 inhibitior	0.74	0.98	0.9412
OCT2 inhibitior	0.85	0.7	0.75
BSEP inhibitior	0.9854	0.9258	0.894
P-glycoprotein inhibitior	0.758	0.9387	0.6479
P-glycoprotein substrate	0.9343	0.5075	0.5338
CYP3A4 substrate	0.7981	0.7262	0.6747
CYP2C9 substrate	1	1	0.7874
CYP2D6 substrate	0.7998	0.7035	0.7654
CYP3A4 inhibition	0.8295	0.8796	0.5457
CYP2C9 inhibition	0.7377	0.9072	0.8084
CYP2C19 inhibition	0.6625	0.9026	0.7191
CYP2D6 inhibition	0.7779	0.8448	0.9417
CYP1A2 inhibition	0.8564	0.8535	0.7347
CYP inhibitory promiscuity	0.5081	0.5054	0.9424
UGT catelyzed	0.6	0	0.7
Carcinogenicity (binary)	0.89	0.79	0.89
Carcinogenicity (trinary)	0.5693	0.5279	0.7104
Eye corrosion	0.9886	0.9886	0.9921
Eye irritation	0.9541	0.9541	0.8602
Ames mutagenesis	0.55	0.99	0.9
Human Ether-a-go-go-Related Gene inhibition	0.6122	0.9363	0.4184
Micronuclear	0.84	0.74	0.86
Hepatotoxicity	0.65	0.9375	0.5851
skin sensitisation	0.8958	0.7115	0.9052
Respiratory toxicity	0.8667	0.9444	0.6556
Reproductive toxicity	0.9889	0.9333	0.7
Mitochondrial toxicity	0.8625	0.9875	0.5125
Nephrotoxicity	0.5342	0.8854	0.845
Acute Oral Toxicity (c)	0.56	0.7852	0.5117
Estrogen receptor binding	0.8907	0.9436	0.8906
Androgen receptor binding	0.8696	0.9487	0.8696
Thyroid receptor binding	0.5487	0.8128	0.5674
Glucocorticoid receptor binding	0.6962	0.7635	0.6814
Aromatase binding	0.6947	0.8655	0.6391
PPAR gamma	0.5862	0.8079	0.7805
Honey bee toxicity	0.7863	0.9541	0.832
Biodegradation	0.725	0.975	0.675
Crustacea aquatic toxicity	0.5	0.54	0.67
Fish aquatic toxicity	0.9498	0.8588	0.9712

pathways that are influenced by irinotecan. Forty molecular pathways associated to colon cancer are now substantially easier to grasp as a direct result of this. The network was designed to demonstrate the connections and interactions that exist between irinotecan and the protein molecules that compose it, between the protein molecules that compose it and the pathways to which they contribute, and between the pathways and the organisms that are ultimately affected by it. In addition, the network was created to demonstrate how the pathways contribute to the protein molecules of irinotecan. Figure 4, which can be seen in [17-19], depicts the intramolecular stability of irinotecan [25-27].

According to the principles of polypharmacology, it is more plausible to suppose that the operation of a single protein-based route requires a large number of different pharmacological molecules than it is to assume that a single chemical can regulate several separate protein-based channels at the same time. In other words, it is more likely to assume that the operation of a single protein-based route requires a large number of different pharmacological molecules. It is more astonishing that a single chemical can affect many distinct protein-based pathways at the same time. This is due to the fact that this has never been done before [28]. When contrasted with the impact of a single target functioning on several routes, the influence of a single route having many protein targets controlled by that molecule can have a large amount of sway over the pathway's overall activity. This is due to the fact that the single pathway encompasses a broader variety of protein targets.

Irinotecan is a type of medication that falls under the category of polypharmacology. The rationale for this classification is that irinotecan has an effect on a large number of proteins and pathways all at the same time [29,30]. The findings that emerged from our investigation brought us to this realization. Irinotecan's effectiveness in combating colon cancer was demonstrated by the formation of a network of protein-pathway links during this study. Irinotecan is able to engage in conversation with the active site domain, as established by the results of an in silico molecular docking investigation. It is possible that this interaction is a crucial component in the network-based mechanisms by which irinotecan inhibits EGFR and other protein molecules. Because of this, the utilization of Irinotecan formulations for the treatment of complex diseases may turn out to be the most viable option [31-33].

CONCLUSION

This study studied Irinotecan's protein targets, gene set enrichment, network analysis, and molecular docking with EGFR. We discovered how Irinotecan alters signalling pathways to limit tumour growth. The study identified irinotecan as a promising colon cancer treatment. Irinotecan mostly affects cancer, metabolic, Ras, and PI3K-Akt signalling pathways. The recent work narrows down Irinotecan's protein targets, which will improve future trials. Irinotecan's poor bioavailability and chemical instability under physiological circumstances prevents its clinical application as an anticancer drug. However, nanoparticle drug delivery technologies may improve Irinotecan bioavailability and

stability. Thus, molecular docking analysis is a fast and cost-effective way to identify the protein targets for distinct anti-cancer drugs from various natural sources and their possible hazardous side effects. In this investigation, we also tested vitamin E TPGS for binding affinity to the permeation glycoprotein and found substantial interaction and binding scores. Based on prior evidence and this investigation, vitamin E TPGS can inhibit efflux pump substrate medicines like irinotecan and others.

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ETHICAL APPROVAL

This study does not involve any animals or human subjects.

CONFLICT OF INTEREST

The authors declare no conflict of interests for this manuscript.

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