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Identification of small molecule inhibitors against doublecortin-like kinase 1 for targeting colon cancer stem cells

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As per the World Health Organisation (WHO) reports, colon cancer ranks second among cancer types affecting women, and third among those affecting men. An evolving hypothesis in the field of oncogenesis posits that a limited population of quiescent cells can give rise to primary tumors. The investigation of cancer stem cells (CSCs) offers a potential avenue for devising innovative approaches to cancer therapy. Notably, the identification of doublecortin-like kinase 1 (DCLK1) assumes significance as it serves as a distinctive marker for CSCs in pancreatic and colon cancer contexts. Nevertheless, the clinical translation of silencing DCLK1 *via* small interfering RNA (siRNA) encounters various pragmatic impediments. Consequently, the pursuit of specific inhibitors targeting DCLK1 emerges as a promising strategy for impeding the processes of cancer initiation, progression, and metastasis, partly by modulating epithelial-mesenchymal transition (EMT) and inducing CSC apoptosis. In this study, our investigation involved querying a repository of traditional Chinese medicinal compounds to discern potential small molecules exhibiting affinity for DCLK1. The compound that displayed the most favorable attributes, luteolin, was subsequently subjected to molecular dynamics simulations. Our computational analysis unveiled luteolin's remarkable qualities, characterized by its conspicuously low binding energy and heightened affinity for the DCLK1 protein. Notably, the simulation divulged a sustained and intricate binding interaction between luteolin and the DCLK1 protein, involving a range of one to four hydrogen bonds throughout the simulation's 100 nanoseconds trajectory. Furthermore, the minimal root mean square deviation observed throughout the simulation duration indicates the stability of the DCLK1-luteolin complex. The identification and validation of distinct inhibitors targeting DCLK1 possess the potential to transform extant approaches to cancer treatment. By exerting control over EMT and instigating the death of CSCs, these inhibitors could aid in transformative changes in the landscape of cancer therapy strategies.

Keywords: Molecular docking, Molecular simulation, Protein-ligand interaction, SwissADME, Traditional chinese medicine database, Virtual screening

According to recent data from the World Health Organization (WHO), the third most common cancer in males and the second most common in women is colorectal cancer. The colon cancer rate in India has increased and the possible reason behind it could be due to changes in diet and following Western diet habits, low levels of physical activity, obesity, extended smoking inflammatory bowel disease, and hereditary[1]. Colorectal cancer has three well-defined stages; initiation changes in the molecular message for a normal cell, and finally promotion and progression these stages finish with phenotypically modified cancer cells[2]. Currently, with the presence of screening and preventive strategies for colon cancer, colorectal cancer remains a significant health issue for people. The present chemotherapy regimens employed for the management of colorectal cancer exhibit notable side effects encompassing symptoms such as diarrhoea, heartburn, organ dysfunction, and muscular cramps[1]. Furthermore, a significant challenge encountered in the therapeutic application of these agents pertains to drug resistance, tumor reappearance, and the advancement of the disease[3]. This predicament can be attributed to the intrinsic properties of a subset of cells residing within the tumor mass, recognized as cancer stem cells (CSCs), which possess attributes such as drug resistance and the capacity for self-renewal. The cancer stem cell hypothesis is an evolving concept of oncogenesis where a few relatively quiescent cells can induce primary tumors. Within a tumor, CSCs are responsible for the initiation progression, tumor metastasis, tumor reappearance, and resistance of

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tumor to cancer therapy (radio- and chemotherapy). Also, CSCs maintain the population in the tumor and replenish and/or support the growth of the tumor heterogenous population *via* two different types of cell division called symmetrical and asymmetrical. With the ability of a tumorigenic cell in the population the identification of specific surface markers for CSCs varies based on the tumor types[4]. Tumorigenesis in the gut the concept begins especially in the base of colonic crypts or nearby situated stem cell populations[5]. Targeting Cancer stem cells (CSCs) as potential therapeutic strategy using potential CSCs-specific markers (Fig. 1).

Recent research has uncovered an intricate association between cancer stem cells (CSCs) and the epithelial-mesenchymal transition (EMT), while historical investigations have established the highly conserved nature of the EMT as a cellular process. Both EMT and CSCs exhibit pronounced implications in tumor advancement and resistance to therapeutic interventions[6]. A notable constituent in this context is doublecortin-like kinase 1 (DCLK1), a microtubule-related kinase that manifests in colon tumors and serves as a putative marker for intestinal stem cells due to its prevalence in post-mitotic neurons. Empirical evidence has indicated that DCLK1-positive CSCs contribute to cancer regression with minimal harm to healthy tissues. Consequently, DCLK1 presents an attractive target for precision cancer therapy, particularly in the domain of colorectal cancer, thereby promising transformative shifts in prevailing cancer treatment paradigms[7]. Recent literature affirms DCLK1's distinctiveness as a marker exclusive to colon cancer, pivotal to colon and pancreatic carcinogenesis murine models. DCLK1 primarily sustains the proliferative capacity of human colon cancer cells. Notably, DCLK1-positive cells display resilience against chemopreventive and chemotherapeutic agents, accentuating the need to therapeutically target DCLK1 to eliminate CSCs and heighten patient survival prospects. DCLK1 also holds prognostic potential as a marker for identifying high-risk colon cancer patients[8]. The underpinnings of intestinal carcinogenesis rest significantly upon DCLK1; its attenuation curtails tumor stemness and progression, allowing modulation of pro-survival signaling and pluripotency. This could be effectively achieved through gene silencing with small interfering RNAs (siRNAs), a potent tool in the RNA interference (RNAi) pathway[9]. While siRNA-mediated gene knockdown presents substantial promise, it is accompanied by practical complexities, impeding its transition to clinical trials for cancer therapy[10]. Notwithstanding the precision of RNAi in gene silencing, its drawbacks encompass off-target effects on normal cells, immune activation, limited cellular uptake, brief half-life, and potential toxicity due to localized siRNA saturation. To circumvent these limitations, alternative strategies such as small molecule inhibitors of kinase have emerged, offering affinity to DCLK1 and inhibitory effects on its activity. These inhibitors exhibit promise in augmenting patient lifespan and therapeutic outcomes. The burgeoning landscape of kinase inhibitors, comprising over 80 agents in diverse clinical trial phases, underscores their potential as novel anticancer therapeutics. Detailed structural elucidation of kinases, such as DCLK1, serves as a

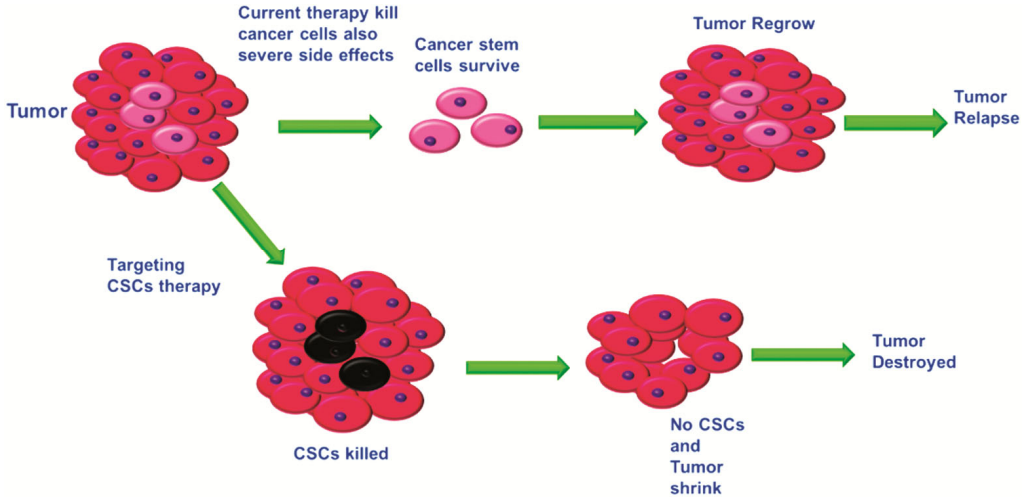


Fig. 1 — Targeting CSCs to eradicate cancer from its root

guide to tailor kinase inhibitors with enhanced efficacy and specificity against intended targets. Our investigation capitalizes on two established binding sites, particularly the ATP-binding site, housing the kinase activation loop in active (type-1) or inactive (type-2) conformations[11]. Evidence underscores the significance of kinase activity inhibition for targeting CSCs, thereby magnifying the potential utility of kinase inhibitors for cancer patients[12]. The pursuit of novel small molecular inhibitors, particularly directed towards cancer cells, holds particular significance for CSCs due to their distinctive properties[13]. Moreover, the integration of traditional Chinese medicine (TCM) into modern therapeutic approaches underscores the importance of identifying active constituents within herbs and elucidating their mechanisms of action. Notably, TCM exhibits promise in suppressing CSC proliferation, self-renewal, multi-differentiation, sphere formation, stemness marker expression, and drug resistance, thereby influencing CSC signaling pathways. The incorporation of TCM-based interventions and inhibitors may offer novel avenues for CSC treatment and catalyze comprehensive exploration into complementary medicinal approaches[14]. Contemporary biological challenges find solutions through *in silico* techniques, furnishing novel avenues for identifying inhibitors against various diseases, including cancer. Molecular docking stands as a conventional methodology to decipher the interactions between ligands and receptors, elucidating compound binding at active sites. This technique aids in discerning binding modes, ligand-receptor interactions, and predicting binding strengths[15,16]. In this study, we unveil novel DCLK1 inhibitors that exhibit the potential to curb cancer initiation, metastasis, and progression by regulating EMT and targeting CSCs. These findings hold substantial promise in revolutionizing contemporary cancer treatment methodologies.

Materials and Methods

Hardware

The *in silico* based investigation was carried out with a Windows 10 platform running on an HP PC with an Intel i5 processor and 8GB of RAM.

Molecular docking analysis

We retrieved the protein structure for DCLK1 from the PDB database (ID: 5JZJ). The subset of the Traditional Chinese Medicine (TCM) database (20,000 compounds) and the natural product ZINC database (15,000 compounds) were used for the virtual screening of potential inhibitors against DCLK1 using the default docking settings offered by the cloud computing web server iScreen (http://iscreen.cmu.edu.tw)[17]. The web server is a combined platform of three systems (the TCM ligand database, docking, and screening unit, and the de novo TCM drug design module).

Molecular dynamic simulation

A molecular dynamic simulation (MDS) was conducted to investigate the stability of both the protein in isolation and the protein-ligand complex when interacting with the top predicted compound. The structure of the DCLK1 complex with the predicted inhibitor (luteolin) was determined by molecular docking of the compounds into the DCLK1 protein (PDB ID: 5JZJ). After this, MDS was executed utilizing GROMACS 5.1.4 software coupled with the GROMOS 53A6 force field. The PRODRG server was harnessed to generate ligand topologies and associated parameters. The resulting data is integrated with the protein topology file to create a simulation complex. A simple point charge (SPC) water model was used as system solvation and the structure of the box was selected as cubic. To equilibrate the counter ions Na^+/Cl^- were added to balance the charges in the system. The system is exposed to temperature stabilization (300K) at the equilibrium phase after energy minimization is accomplished. The NPT ensemble then used the MDS to maintain the pressure at 1 atm. Following the equalization of the temperature and pressure, a production molecular dynamic simulation was run for 100 ns. For the DCLK1 isolated and DCLK1 - luteolin complex, final MD trajectories were studied using inbuilt GROMACS trajectory analysis commands. The stability of the complex was studied by root mean square fluctuation (RMSF), root mean square deviation (RMSD), and protein-ligand interaction with amino acids was studied by a hydrogen bond (H-bond) analysis[18].

Determining ADMET properties

The investigated compounds were translated into SMILES format using SMILES Translator Online Help. They were then submitted to SwissADME for ADMET analysis, physicochemical parameter prediction, and drug-likeness using the Lipinski rule of 5. Lipinski's Rule of Five established the link between pharmacokinetic and physicochemical parameters[19].

Table 1 — Top six potential ligands for DCLK1 (kinase domain) obtained from virtual screening of TCM database

Rank	TCM database ligand ID	Ligand Name	Score	Interacting amino acids *via* hydrogen bonding
1	5016	luteolin	-83.8963	VAL468, GLU 466
2	2358	3,3'4'5,7-pentahydroxyflavanone	-82.2119	VAL468, GLU 466, ASP 533
3	2845	Swerchirin	-81.5518	GLU 466, ASP 533
4	2333	23-dihydrofisetin	-80.5123	VAL468, GLU 466
5	27	Acacetin	-80.1762	VAL468, GLU 466
6	7997	4-Methoxy-7-oxofuro(3,2-g)Chromen-(9-yl) acetate	-79.6584	VAL468, ASP 472

Results

Virtual screening and docking

Initially, the top 200 ligand hits were selected based on their higher negative score which implies better binding affinity. Subsequently, these top 200 interacting ligand hits along with their 4Å vicinity were analyzed with PyMOL (Version 2.4) to understand the hot spot details of interacting residues with the protein target DCLK-1. Finally, the top six ligands as potential inhibitors for DCLK-1 (kinase domain) were chosen from the virtual screening approach performed using the iScreen web server in this study[18]. The protein-ligand interacting residues and the six small-molecule ligand details are tabulated below (Table 1).

Anticancer and anti-CSC activities of the identified compounds

Luteolin primarily suppresses tumourgrowth by deactivating several signals and transcriptional pathways important for cancer cells. By phosphorylating JNK and activating the mitochondrial pathways of apoptosis while suppressing NF-B translocation, luteolin can cause apoptosis in human non-small-cell lung cancer A549[19]. Luteolin dramatically reduced tumour growth, decreased cell proliferation, and promoted apoptosis in a nude mouse H460 xenograft tumour model. Application of luteolin to tumour tissues led to a significant increase in miR-34a-5p, as confirmed by quantitative PCR (qPCR) and miRNA microarray analyses.In non-small-cell lung cancer cells and tumour tissues, luteolin therapy was linked to elevated p53 and p21 protein levels and decreased MDM4 protein expression[20]. A combination of direct and indirect mechanisms prevents metastases when luteolin is used. As an antiangiogenic therapy, luteolin, for instance, may prevent the invasion of breast cancer by preventing the synthesis of VEGF and the activity of its receptor. The indicators of the epithelial-mesenchymal transition and the propensity for metastatic growth are also decreased by luteolin. By inhibiting receptor tyrosine-kinase activity and apoptosis, which both potentially stop breast cancer from initially colonizing cells, luteolin also has antiproliferative properties[21]. These gastric cancer cells were treated with luteolin, which greatly decreased STAT3 phosphorylation and decreased the expression of the genes Mcl-1, Surviving, and Bcl-xl that target STAT3. The protein tyrosine phosphatase SHP-1, whose silencing eliminated luteolin's inhibitory effect on STAT3 and cell death, may be essential for luteolin-mediated cellular activity[22]. By encouraging antioxidant activity and stimulating MAPK signaling in human colon cancer cells, luteolin causes apoptosis[23]. Lutein suppresses the stemness of prostate cancer cells by upregulating FZD6 transcriptionally, which inhibits WntSignaling. Additionally, we found that upregulating FZD6 inhibits Wnt signaling, which is a mechanism underlying the luteolin-induced decrease of prostate cancer stemness. FZD6 is a tumour suppressor that can eliminate prostate cancer stemness[24]. Luteolin did not significantly affect the cytotoxicity of healthy epithelial cells, but it successfully reduced the rate of proliferation, self-renewal, aldehyde dehydrogenase 1 activity, and CD44-positive oral cancer stem cells. Additionally, luteolin returned the oral cancer stem cells' radiosensitivity[25]. Swerchirin showed an IC_{50} of 20 M at 48 h of incubation and dose-dependently decreased the cell viability in human ovarian cancer cells. Swerchirin's anticancer effect was discovered to be caused by mitochondrial apoptosis and G2/M cell cycle arrest[26]. Acacetin can prevent the phosphorylation of p38 MAPK, which is necessary for the downregulation of the expression of urokinase-type plasminogen activator (u-PA), matrix metalloproteinase-2 (MMP-2), and matrix metalloproteinase-9 (MMP-9), in human prostate cancer cells[27]. In human prostate cancer cells, acacetin caused a greater, time- and dose-dependent reduction of cell proliferation (20–70%) that was followed by

cell death. According to the doses and treatment durations, acacetin demonstrated a stronger G1 and/or G2-M arrest. Cip1/p21 levels rose while CDK2, CDK4, and CDK6 protein concentrations fell in response to G1 arrest[28].With no apparent side effects, acacetin shown enhanced the regression of gastric cancer xenograft tumours *in vivo* by lowering the protein levels of pEGFR in tumours[29] (Table 2).

Molecular docking

Globally, the TCM database is data mined to identify new novel cancer inhibitors. Artemisinin, an antimalarial drug from TCM has been recently identified as the origin for the synthesis of several anti-cancer compounds like artesunate and other sesquiterpene lactone-based drugs. This provided the impetus for us to use it as a promising approach for identifying potential anticancer therapeutics against DCLK1. Virtual screening is a technique used for identifying potential ligands that can interact with a target molecule. In this study, we identified six potential ligands that can interact with DCLK1. Among them, swerchirin, luteolin, and acacetin have the best binding affinity (Table 1) against the DCLK1 protein. Figure 2A-2F shows the binding cavity, the ligand, and its interaction vicinity within the 4Å of the active site region for the chosen six compounds.

Table 2 — Earlier Reports on anticancer and anti-CSC activities of the Identified Compounds

Ligand Name	Targets	Cancer types	References
Luteolin	JNK	Non-small cell lung cancer	(19)
	MicroRNA-34a-5p	Non-small cell lung cancer	(20)
	VEGF	Breast cancer	(21)
	STAT3	Gastric cancer	(22)
	MAPK	Colon cancer	(23)
	Wnt	Prostate cancer	(24)
	ALDH1, CD44	Oral cancer stem cell	(25)
Swerchirin	G2/M cell cycle arrest	Human ovarian cancer cells	(26)
Acacetin	p38 MAPK	Human prostate cancer	(27)
	CDK2, CDK4 and CDK6	prostate Cancer	(28)
	Akt/NF-κBpEGFR	Prostate cancerProstate cancer	(29)

Molecular dynamic simulation

From the top hits of the compounds from the molecular docking studies, luteolin is selected further to perform the molecular dynamic simulation and evaluate the stability of the compound in the binding pocket of the DCLK1 protein (Fig. 3A-3C). We found that the luteolin ligand was stable throughout the simulation and did not eject out from the binding pocket of the DCLK1 protein. Further, the RMSF graph also shows the minimal fluctuation of the protein residues located at 460-470. This minimum fluctuation will result in the reduction of delta-G and promote stronger bond interaction between the DCLK1 protein and luteolin ligand. Although there have been some effects on the protein residues due to their interaction with a ligand, molecule, the overall protein is stable after the 60ns MDS run. This further confirms the protein-ligand complex is stable and has strong interaction throughout the simulation run. Further, the RMSD of the DCLK1-luteolin complex ranged from 0.2 to 0.6 nm, at the end of the simulation, the RMSD did not fluctuate more and was in the fine range of 0.35 to 0.4 nm, showing it stabilizing from 60 ns of the simulation till the end of the MDS *i.e.* 100 ns.DLCK1-luteolin is stable during 100ns molecular dynamic simulation run with actively interacting with the active site amino acid residues. Further, complementing the molecular docking results the luteolin makes the same hydrogen bond with the DCLK1 protein at VAL 468 and GLU 466 while keeping one hydrogen bond throughout the simulation process in the active site (binding pocket).

ADMET parameters

To develop a drug, computer-aided drug design requires pre-clinical optimization of physicochemical properties, absorption, distribution, metabolism, and excretion (ADME), as well as *in silico* toxicity evaluation. The pharmacokinetic profile of a chemical determines the IUPAC name, canonical smiles its absorption, distribution, metabolism, and excretion (ADME) (Tables 3 and 4).

Discussion

Cancer aggression, recurrence, and medication resistance are all influenced by cancer CSCs. Targeting CSC markers or genes can improve the efficiency of current cancer treatment strategies[30]. We can use strategies to eliminate the CSCs from tumor mass to target the surface-specific markers, mechanisms, and proteins involved in CSC

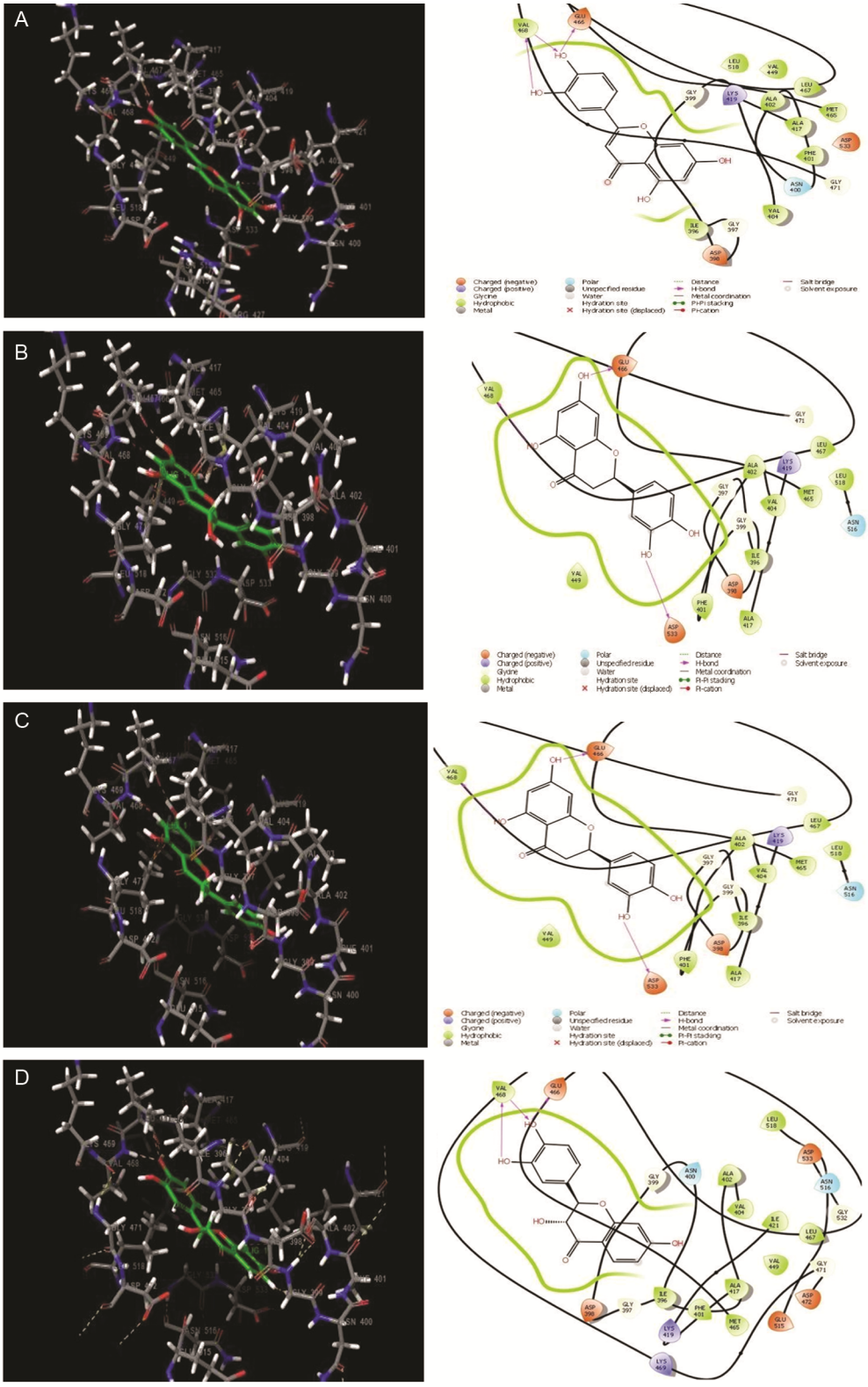
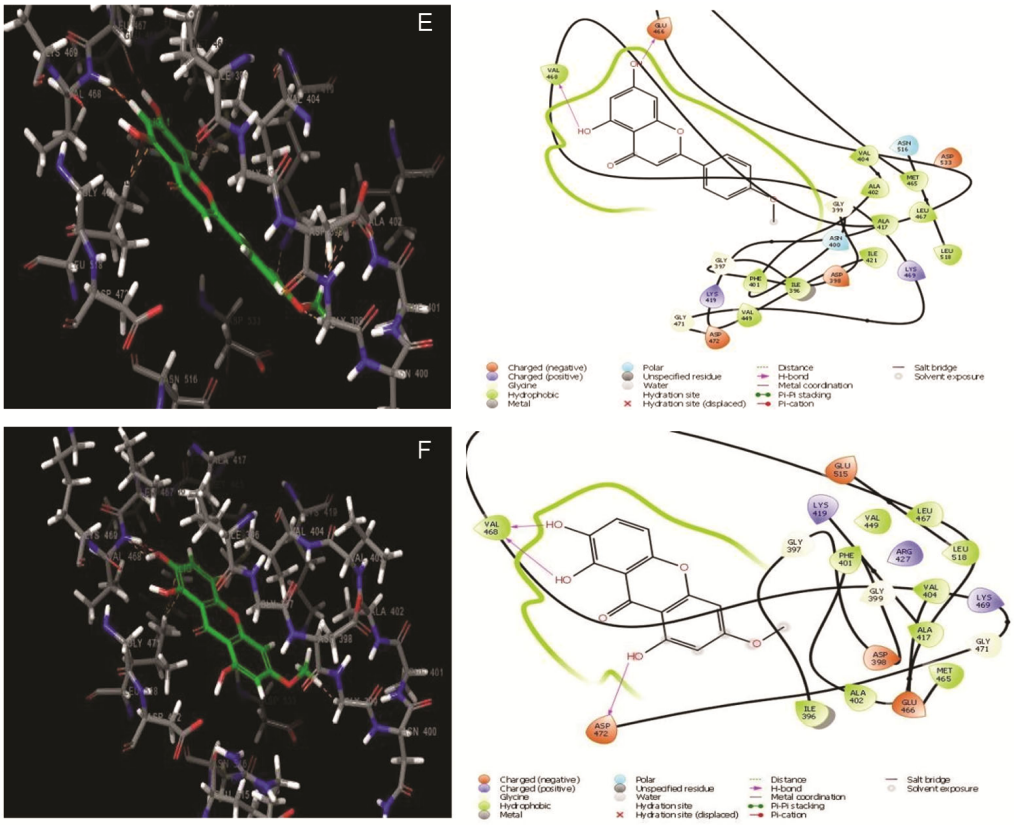


Fig. 2 — The binding cavity of ligand and Ligand interaction map for TCM database ligand ID: (A) 5016-luteolin; (B) 2358-3,3'4'5,7-pentahydroxyflavanone; (C) 2845- Swerchirin; (D) 2333- 23-dihydrofisetin; (E) 27- Acacetin; and (F) 7997- 4-Methoxy-7-oxofuro (3, 2-g)Chromen-(9-yl)acetate



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maintenance, drug resistance, and signaling pathways that sustain CSC growth[4]. Studies have shown that a small-molecule kinase inhibitor has been an effective inhibitor of CSC activation[12]. Protein kinases are referred to by their ability to catalyze the exchange of terminal phosphate of ATP to a substrate that generally contains an amino acid residue such as serine, threonine, or tyrosine so the kinase inhibitors could be targeting the ATP binding site[11]. Several reports show that DCLK 1 is a CSC marker for colon cancer and targeting the DCLK1 could change in current cancer treatments[7]. Studies report that virtual screening and docking-based studies for the identification of inhibitors to target cancer or ligand-based drug discovery for cancer will provide a therapeutic application[15]. DCLK1 belongs to the group of microtubule-binding proteins of calmodulin-dependent kinase, and it has been proposed as the stem cell marker for the pancreas and intestine. DCLK 1 expression is found in the Apc (Min/+) adenomas[31] and using lineage-tracing methods, reports have shown that Dclk1 no longer marks normal stem cells within the intestine however alternatively mark only CSCs that always results in the polyp's production in the Apc (Min/+) mice and DCLK1 is the ability to a specific marker for targeting colon CSCs. Studies suggested that overexpression of DCLK 1 in colon cancer tissue and very low in the normal colon tissue and the specimen with stain was specifically impressive in the stroma surrounding malignant crypts. These results suggest an interaction between epithelial cell populations and have an important role in assisting their proliferation and communication between stromal cells and epithelial stem cells[7].

Here, we used the TCM docking database to identify the small molecule inhibitors for the DCLK1 kinase domain. The potential inhibitors of DCLK1 were identified using the Schrödinger - Glide module. The structure of the molecules has been manually obtained from the PubChem database. Clinical experiments suggested that traditional Chinese medicine plays an important role in the treatment of cancer and its prevention. Extracts of Chinese herbals indicate powerful inhibitory effects against carcinogenesis and

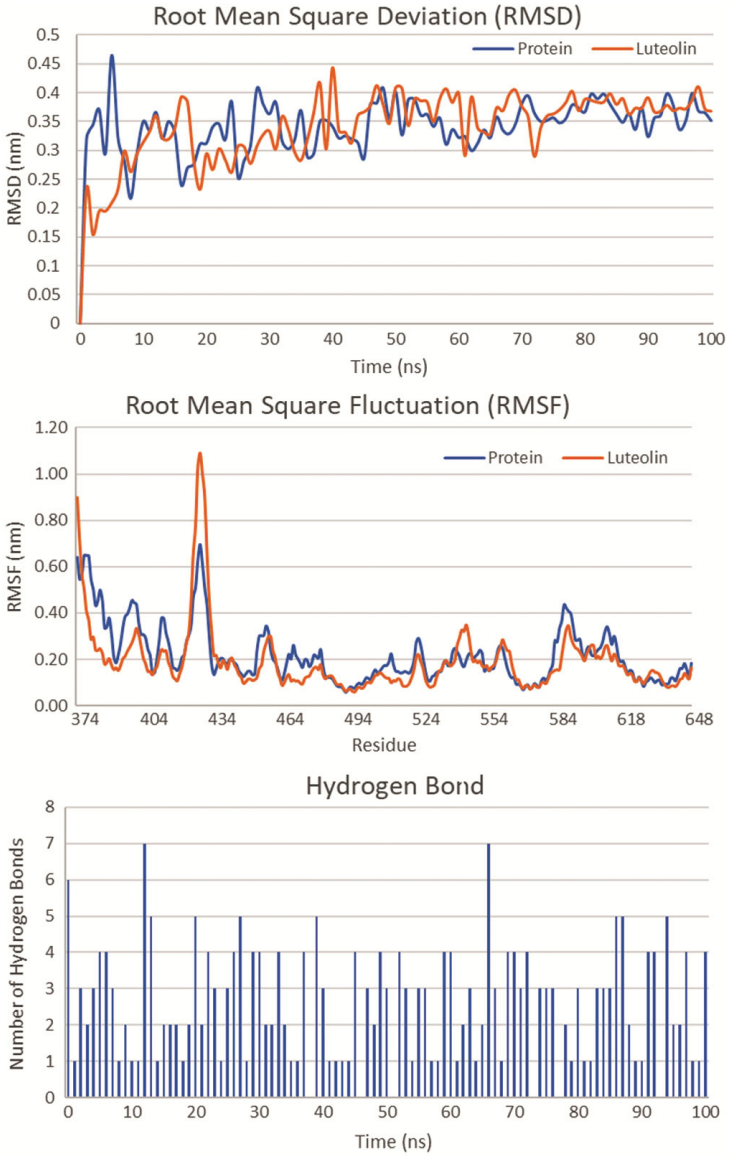

Fig. 3 — The Protein (DCLK1) and the luteolin (DCLK1-luteolin) complex's molecular dynamic simulation trajectory analysis during the 100-ns simulation is shown in (A) RMSD; (B) RMSF; and (C) hydrogen bonding

Table 3 — IUPAC name and SMILES code for all six potential ligands for DCLK1

Sr. No	IUPAC Name	Canonical Smiles
1	2-(3,4-dihydroxyphenyl)-5,7-dihydroxychromen-4-one	C1=CC(=C(C=C1C2=CC(=O)C3=C(C=C(C=C3O2)O)O)O)O
2	(2R,3R)-2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-2,3-dihydrochromen-4-one	C1=CC(=C(C=C1C2C(C(=O)C3=C(C=C(C=C3O2)O)O)O)O)O
3	1,8-dihydroxy-3,5-dimethoxyxanthen-9-one	COC1=C2C(=C(C=C1)O)C(=O)C3=C(C=C(C=C3O2)OC)O
4	(2R,3R)-2-(3,4-dihydroxyphenyl)-3,7-dihydroxy-2,3-dihydrochromen-4-one	C1=CC(=C(C=C1C2C(C(=O)C3=C(O2)C=C(C=C3)O)O)O)O
5	5,7-dihydroxy-2-(4-methoxyphenyl)chromen-4-one	COC1=CC=C(C=C1)C2=CC(=O)C3=C(C=C(C=C3O2)O)O
6	[(2R)-3-hydroxy-1-(4-methoxy-7-oxofuro[3,2-g]chromen-9-yl)oxy-3-methylbutan-2-yl] acetate	CC(=O)OC(COC1=C2C(=C(C3=C1OC(=O)C=C3)OC)C=CO2)C(C)(C)O

Table 4 — *In silico* computed physicochemical characteristics for all six potential ligands of DCLK1

Molecule	Canonical SMILES	Formula	MW	MR	TPSA	I LOGP	Ali Class	GI absorption	BBB permeant	Bioavailability Score
Luteolin	Oc1cc(O)c2c(c1)oc(cc2=O)c1ccc(c(c1)O)O	C15H10O6	286.24	76.01	111.13	1.86	Moderately soluble	High	No	0.55
3,3′4′5,7-pentahydroxyflavanone	Oc1ccc2c(c1)OC(C(C2=O)O)c1cc(O)c(c(c1)O)O	C15H12O7	304.25	74.76	127.45	0.8	Soluble	High	No	0.55
Swerchirin	COc1cc(O)c2c(c1)oc1c(c2=O)c(O)ccc1OC	C15H12O6	288.25	77.02	89.13	2.9	Moderately soluble	High	No	0.55
23-dihydrofisetin	Oc1ccc2c(c1)OC(C(C2=O)O)c1ccc(c(c1)O)O	C15H12O6	288.25	72.73	107.22	1.47	Soluble	High	No	0.55
Acacetin	COc1ccc(cc1)c1cc(=O)c2c(o1)cc(cc2O)O	C16H12O5	284.26	78.46	79.9	2.56	Moderately soluble	High	No	0.55
4-Methoxy-7-oxofuro (3, 2-g) Chromen-(9-yl) acetate	COc1c2ccoc2c(c2c1ccc(=O)o2)OCC(C(O)(C)C)OC(=O)C	C19H20O8	376.36	96.57	108.34	3.23	Moderately soluble	High	No	0.55

regulate the cancer pathways such as NF-κB, MAPK, and JAK/STAT[32]. From our results, we obtained six ideal inhibitors based on the score value for DCLK 1 to target the colon cancer stem cells from the TCM database. Based on the binding affinity towards ATP compounds were obtained from the Schrödinger-Glide module. Studies have shown targeting the activity of kinase by appropriate inhibitors can target the survival of cancer and/or proliferation. Although of most kinase inhibitors do not utilize the ribose binding site of ATP's triphosphate binding site. One such important ligand that is necessary for the operation of many proteins and functions as a coenzyme is ATP[33] which targets the kinase domain by the ATP binding site for targeting cancer treatment[34]. In this study, we have shown six very effective inhibitors to the kinase domain for DCLK 1 interact with the active region of ligands amino acids such as VAL468, GLU 466, and ASP 533 *via* hydrogen bonding. From these inhibitors following compounds acacetin, luteolin and swerchirin have been shown to target cancer and cancer stem cells. Our results suggest that inhibiting DCLK1 can reduce colon cancer initiation, progression, and metastasis of colon cancer *via* EMT, and eradication of CSCs. Identifying novel small molecule inhibitors from naturally occurring compounds with low toxicity that are probably used chemotherapeutically in the treatment and preventive methods for cancer. Swiss ADMET analysis suggests that our six compounds have a positive therapeutic profile and are safe, with minor drawbacks. These optimistic results contribute to the development and confirmation of unique DCLK1 inhibitors that have the potential to revolutionize current colon cancer treatment therapy.

Conclusion

We concluded from the literature, the knockdown of the DCLK1 expression will reduce tumor growth in a xenograft model. This study suggested that DCLK1, a cancer stem cell marker, can be a potential therapeutic target for colon cancer treatment. From all the docking hits compounds such as luteolin, acacetin, and swerchirin have already shown anticancer activity in various cancer types. Luteolin and acacetin have shown anti-cancer stem cell activity in different cancer types. The molecular dynamic simulation also shows a constant hydrogen bonding between luteolin and DCLK1 binding pocket, as well as the ligand-receptor complex, which was stable throughout the 100 ns simulation. The RMSD fluctuation throughout the simulation was also minimal throughout the simulation time. Suggesting the stability of ligands with the receptor-binding site. SwissADME results suggest that our compounds follow Lipinski rule 5 do not cross the brain barrier and have good solubility. From our findings, the identified small-molecule inhibitors from the TCM database to target the colon cancer stem cells using DCLK1 could help in eradicating colon cancer from its roots. Further *in vitro* and *in vivo* studies can be conducted to understand more about the inhibitory effect of luteolin on DCLK1 expression and regression of colon cancer stem cells.

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Conflict of interest

All authors declare no conflict of interest.

References

1 Lazer LM, Sadhasivam B, Palaniyandi K, Muthuswamy T, Ramachandran I, Balakrishnan A, Pathak S, Narayan S & Ramalingam S, Chitosan-based nano-formulation enhances the anticancer efficacy of hesperetin. *Int J Biol Macromol*, 107 (2018) 1988.

2 Simon K, Colorectal cancer development and advances in screening. *Clin Interv Aging*, 11 (2016) 967.

3 Phi LT, Sari IN, Yang YG, Lee SH, Jun N, Kim KS, Lee YK & Kwon HY, Cancer stem cells (CSCs) in drug resistance and their therapeutic implications in cancer treatment. *Stem Cells Int*, 1 (2018) 5416923.

4 Dragu DL, Necula LG, Bleotu C, Diaconu CC & Chivu-Economescu M, Therapies targeting cancer stem cells: Current trends and future challenges. *World J Stem Cells*, 7 (2015) 1185.

5 Ponnurangam S, Mammen JM, Ramalingam S, He Z, Zhang Y, Umar S, Subramaniam D & Anant S, Honokiol in combination with radiation targets notch signaling to inhibit colon cancer stem cells. *Mol Cancer Ther*, 11 (2012) 963.

6 Liu X & Fan D, The epithelial-mesenchymal transition and cancer stem cells: functional and mechanistic links. *Curr Pharm Des*, 21 (2015) 1279.

7 Nakanishi Y, Seno H, Fukuoka A, Ueo T, Yamaga Y, Maruno T, Nakanishi N, Kanda K, Komekado H, Kawada M & Isomura A, Dclk1 distinguishes between tumor and normal stem cells in the intestine. *Nat Genet*, 45 (2013) 98.

8 Sureban SM, May R, Weygant N, Qu D, Chandrakesan P, Bannerman-Menson E, Ali N, Pantazis P, Westphalen CB, Wang TC & Houchen CW, XMD8-92 inhibits pancreatic tumor xenograft growth *via* a DCLK1-dependent mechanism. *Cancer Lett*, 351 (2014) 151.

9 Weygant N, Qu D, Berry WL, May R, Chandrakesan P, Owen DB, Sureban SM, Ali N, Janknecht R & Houchen CW, Small molecule kinase inhibitor LRRK2-IN-1 demonstrates potent activity against colorectal and pancreatic cancer through inhibition of doublecortin- like kinase 1. *Mol Cancer*, 13 (2014) 1.

10 Dorsett Y & Tuschl T, siRNAs: applications in functional genomics and potential as therapeutics. *Nat Rev Drug Discov*, 3 (2004) 318.

11 Zhang J, Yang PL &Gray NS, Targeting cancer with small molecule kinase inhibitors. *Nat Rev Cancer*, 9 (2009) 28.

12 Tan X, Yan Y, Song B, Zhu S, Mei Q & Wu K, Focal adhesion kinase: from biological functions to therapeutic strategies. *Exp Hematol Oncol*, 12 (2023) 83.

13 Park JH, Chung S, Matsuo Y & Nakamura Y, Development of small molecular compounds targeting cancer stem cells. *Medchemcomm*, 8 (2017) 73.

14 Tang KY, Du SL, Wang QL, Zhang YF & Song HY, Traditional Chinese medicine targeting cancer stem cells as an alternative treatment for hepatocellular carcinoma. *J Integr Med*, 18 (2020) 196.

15 Ganeshpurkar A, Chaturvedi A, Shrivastava A, Dubey N, Jain S, Saxena N, Gupta P & Mujariya R, *In silico* interaction of Berberine with some immunomodulatory targets: A docking analysis. *Indian J Biochem Biophys*, 59 (2022) 8.

16 Choudhury M, Sharma D, Das M & Dutta K, Molecular docking studies of natural and synthetic compounds against human secretory PLA2 in therapeutic intervention of inflammatory diseases and analysis of their pharmacokinetic properties. *Indian J Biochem Biophys*, 59 (2022) 1.

17 Singh N, Chaput L & Villoutreix BO, Virtual screening web servers: designing chemical probes and drug candidates in the cyberspace. Briefings in bioinformatics. 2 (2021) 1790.

18 Latha V, Gomathi V & Rajeshkanna A, Generating a potent inhibitor against MCF7 breast cancer cell through artificial intelligence based virtual screening and molecular docking studies. *Indian J Biochem Biophys*, 60 (2023) 11.

19 Tuorkey MJ, Molecular targets of luteolin in cancer. *Eur J Cancer Prev*, 25 (2016) 65.

20 Jiang ZQ, Li MH, Qin YM, Jiang HY, Zhang X & Wu MH, Luteolin inhibits tumorigenesis and induces apoptosis of non-small cell lung cancer cells *via* regulation of MicroRNA-34a-5p. *Int J Mol Sci*, 19 (2018) 447.

21 Lin D, Kuang G, Wan J, Zhang X, Li H, Gong X & Li H, Luteolin suppresses the metastasis of triple-negative breast cancer by reversing epithelial-to-mesenchymal transition *via* downregulation of β-catenin expression. *Oncol Rep*, 37 (2017) 895.

22 Song S, Su Z, Xu H, Niu M, Chen X, Min H, Zhang B, Sun G, Xie S, Wang H & Gao Q, Luteolin selectively kills STAT3 highly activated gastric cancer cells through enhancing the binding of STAT3 to SHP-1. *Cell Death Dis*, 8 (2017) 2612.

23 Kang KA, Piao MJ, Ryu YS, Hyun YJ, Park JE, Shilnikova K, Zhen AX, Kang HK, Koh YS, Jeong YJ & Hyun JW, Luteolin induces apoptotic cell death *via* antioxidant activity in human colon cancer cells. *Int J Oncol*, 51 (2017) 1169.

Singh RP, Agrawal P, Yim D, Agarwal C & Agarwal R, Acacetin inhibits cell growth and cell cycle progression, and induces apoptosis in human prostate cancer cells: structure-activity relationship with linarin and linarin acetate. *Carcinogenesis*, 26 (2005) 845.

24 Tu DG, Lin WT, Yu CC, Lee SS, Peng CY, Lin T & Yu CH, Chemotherapeutic effects of luteolin on radio-sensitivity enhancement and interleukin-6/signal transducer and activator of transcription 3 signaling repression of oral cancer stem cells. *J Formos Med Assoc*, 115 (2016) 1032.

25 Luo L, Zhao H & Luo Q, Swerchirin exerts anticancer activity on SKOV3 human ovarian cancer cells via induction of mitochondrial apoptosis, G2/M cell cycle arrest and inhibition of Raf/MEK/ERK cascade. *JBUON*, 23 (2018) 111.

26 Shen KH, Hung SH, Yin LT, Huang CS, Chao CH, Liu CL & Shih YW, Acacetin, a flavonoid, inhibits the invasion and migration of human prostate cancer DU145 cells *via* inactivation of the p38 MAPK signaling pathway. *Mol Cell Biochem*, 333 (2010) 279.

27 Jeong SH, Kim HH, Park MY, Bhosale PB, Abusaliya A, Won CK, Park KI, Kim E, Heo JD, Kim HW & Ahn M, Flavones: The apoptosis in prostate cancer of three flavones selected as therapeutic candidate models. *Int J Mol Sci*, 24 (2023) 11.

28 Kim HR, Park CG & Jung JY, Acacetin (5, 7-dihydroxy-4'-methoxyflavone) exhibits *in vitro* and *in vivo* anticancer activity through the suppression of NF-κB/Akt signaling in prostate cancer cells. *Int J Mol Med*, 33 (2014) 317.
29 Li Y, Wang Z, Ajani JA & Song S, Drug resistance and Cancer stem cells. *Cell Commun Signal,* 19 (2021) 1.
30 Chandrakesan P, Yao J, Qu D, May R, Weygant N, Ge Y, Ali N, Sureban SM & Gude M, Vega K, Bannerman-Menson E, Dclk1, a tumor stem cell marker, regulates pro-survival signaling and self-renewal of intestinal tumor cells. *Mol Cancer*, 16 (2017) 30.
31 Jiao L, Bi L, Lu Y, Wang Q, Gong Y, Shi J & Xu L, Cancer chemoprevention and therapy using chinese herbal medicine. *Biol Proced Online*, 20 (2018) 1.
32 Chauhan JS, Mishra NK & Raghava GP, Identification of ATP binding residues of a protein from its primary sequence. *BMC Bioinformatics*, 10 (2009) 434.
33 Bhullar KS, Lagarón NO, McGowan EM, Parmar I, Jha A, Hubbard BP & Rupasinghe HPV, Kinase-targeted cancer therapies: progress, challenges and future directions. *Mol Cancer*, 17 (2018) 48.