Investigation of scutellarin's mechanism in inhibiting colon cancer cells through molecular docking analysis

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Abstract:

Colon cancer, also referred to as colorectal cancer when involving both the colon and rectum, originates in the large intestine. Alongside conventional treatments, targeted therapy has garnered increasing attention. The wnt/β-catenin pathway is one of the therapeutic targets under investigation for colon cancer. Scutellarin, a bioactive compound, has demonstrated efficacy in cancer treatment across numerous studies. This study evaluates the impact of scutellarin on three key targets within the wnt/β-catenin pathway using molecular docking techniques and analyses its drug-likeness characteristics, pharmacokinetics, and toxicity parameters. Results indicate that scutellarin inhibits the wnt/β-catenin pathway, evidenced by its significantly low binding energy values with three proteins: catenin beta-1, glycogen synthase kinase-3 beta, and casein kinase I isoform delta. Pharmacokinetic predictions suggest scutellarin exhibits poor intestinal absorption, good tissue distribution, does not cross the blood-brain barrier, is metabolised in the liver, excreted via the kidneys, and displays no toxicity. Lipinski's rule analysis confirms that scutellarin possesses drug-like properties. Consequently, further *in vitro* and *in vivo* studies are warranted to comprehensively evaluate its potential in colon cancer treatment.

Keywords: **colon cancer, *in silico*, molecular docking, scutellarin, wnt/β-catenin pathway.**

Classification numbers: **3.2, 3.3, 3.6**

1. Introduction

Colon cancer is characterised by the abnormal growth of cells in the tissues of the colon or rectum and is often referred to as colorectal adenocarcinoma. This cancer typically arises from the glandular epithelial cells of the colon. It develops when cells in the epithelial tissue undergo a series of genetic or epigenetic mutations, facilitating uncontrolled growth [1]. According to Globocan 2020, colorectal cancer ranks third globally in incidence, following lung and breast cancers, and second in mortality [2]. Standard treatments include surgery, chemotherapy, and radiation therapy. However, due to high rates of late-stage detection and recurrence, these treatments often yield limited prognoses and are accompanied by significant side effects. Therefore, developing more definitive and comprehensive therapeutic approaches is essential.

Targeted therapies and anti-angiogenesis treatments, such as monoclonal antibodies and small molecule drugs, have shown considerable promise, particularly for patients with advanced or metastatic colon cancer, where localised treatments like surgery and radiation are not feasible. These therapies inhibit tumour growth and metastasis by blocking specific signalling pathways, offering an effective treatment alternative.

Large-scale genomic sequencing has identified wnt/β-catenin signalling as a predominant oncogenic driver in colon cancer, with mutations in genes encoding pathway components detected in over 80% of patients [3]. This pathway comprises a family of proteins critical for embryonic development and tissue homeostasis. It consists of four segments: extracellular, membrane, cytoplasmic, and nuclear. Dysregulation of wnt/β-catenin signalling is implicated in various severe diseases, including cancers and non-cancerous conditions [4].

The wnt/β-catenin pathway regulates β-catenin, a pivotal protein in gene transcription and cell cycle initiation. Under normal conditions, β-catenin is bound and regulated by a multiprotein complex including axin, APC (Adenomatous polyposis coli), GSK3 (Glycogen synthase kinase 3 beta), and CK1 (Casein kinase I). This complex phosphorylates β-catenin, targeting it for proteasomal degradation, thereby maintaining low cellular levels. Upon wnt signal binding to cell surface receptors, this regulatory complex is disrupted, specifically recruiting axin, APC, GSK3, and CK1 to the

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wnt receptor complex. This recruitment inhibits β-catenin phosphorylation, allowing it to accumulate in the cytoplasm. Accumulated β-catenin subsequently translocates to the nucleus, where it interacts with transcription factors such as LEF1 (Lymphoid enhancer-binding factor 1) and TCF (T-cell factor), thereby stimulating the transcription of target genes that promote cell proliferation [4].

In colon cancer, wnt/β-catenin signalling is primarily driven by the abnormal cytoplasmic accumulation of β-catenin, which then translocates to the nucleus. There, it forms a transcriptional complex with TCF, leading to the activation of wnt target gene transcription [5]. The aberrant accumulation of β-catenin results from the inhibition of its repressive complex, comprising axin, GSK3β, CK1D, and APC, which prevents its phosphorylation and degradation. Nuclear β-catenin interacts with TCF and LEF, along with co-activators such as pygopus and Bcl-9, activating wnt pathway target genes, including c-Myc, cyclin D1, and CDKN1A. This dysregulation promotes abnormal cell proliferation and tumour progression [6].

Currently, no drugs have been officially developed and approved that specifically target the wnt/β-catenin pathway for cancer treatment. Scutellarin, a compound extensively studied for its efficacy in cancer therapy, has been shown to inhibit glioma cell growth by regulating BIRC5 reduction to promote apoptosis [7]. It has demonstrated effectiveness against colorectal cancer through gene therapy [8] and notably inhibits gastric cancer cell progression by targeting the wnt/β-catenin pathway, thereby enhancing apoptosis [9].

Scutellarin is a natural flavonoid recognised for its multiple biological effects, including neuroprotective, antibacterial, and antiviral properties. In addition to these functions, scutellarin exhibits antitumour properties. Its underlying antitumour mechanism involves the inhibition of various signalling pathways, such as the Janus kinase/signal transducers and activators of transcription (Jak/STAT), extracellular signal-regulated kinase/AMP-activated protein kinase (ERK/AMPK), and wnt/β-catenin. Furthermore, scutellarin activates both intrinsic and extrinsic apoptosis pathways, leading to tumour cell death, disrupting the cell cycle, and promoting cell cycle arrest [10]. This study focuses on elucidating the antitumour effects of scutellarin on the wnt/β-catenin pathway and examining its pharmacokinetic properties.

Molecular docking is a computational technique used to predict and simulate molecular interactions, specifically assessing the binding affinity between proteins and other molecular structures. It determines the optimal configuration, spatial orientation, and stability of complexes at their lowest energy levels [11, 12]. This method enhances research efficiency, reduces costs, and facilitates the screening of potential compounds and the design of novel drugs. In this study, we assess the inhibitory potential of scutellarin on three key targets in the wnt/β-catenin pathway through molecular docking and evaluate its drug-likeness, pharmacokinetics, and toxicity parameters.

2. Materials and methods

2.1. Molecular docking

Preparation of protein structures: The 3D structures of the target proteins were retrieved from the Protein Data Bank (PDB) (https://www.rcsb.org): catenin beta-1 (PDB ID: 7AFW), glycogen synthase kinase-3 beta (PDB ID: 3ZDI), and casein kinase I isoform delta (PDB ID: 4TN6). Each structure contained its respective crystallised ligand: R9Q (3-[(2-[1])-4-methyl-5-oxidanylidene-2,3-dihydro-1,4-benzoxazepin-2-yl]benzenecarbonitrile), UGJ (3,6-diamino-4-(2-chlorophenyl)thieno[2,3-b]pyridine-2,5-dicarbonitrile), and PFO (4-[4]pyrimidin-2-amine).

The active sites of the proteins were identified using Discovery Studio Visualizer 4.0 software. Water molecules and crystallised ligands were removed, and hydrogen atoms were added using AutoDock Vina software. The active site positions were reset using Molecular graphics lab (MGL) AutoDock Tools 1.5.7 software, as shown in Table 1.

Table 1. The active sites of three important protein targets.

ID Protein Data Bank	Grid box size (Å)	Grid spacing	(XYZ)
7AFW	32x32x32	0.375	(61; -44; 20)
3ZDI	30x30x30	0.375	(-9; -6; 8)
4TN6	60x60x60	0.378	(-21; -2; 39)

Preparation of ligand structures: The 3D structure of scutellarin, with the International Union of Pure and Applied Chemistry (IUPAC) name (2S,3S,4S,5R,6S)-6-[5,6-dihydroxy-2-(4-hydroxyphenyl)-4-oxochromen-7-yl]oxy-3,4,5-trihydroxyoxane-2-carboxylic acid, was retrieved from the PubChem database (https://pubchem.ncbi.nlm.nih.gov) in SDF format and converted to PDB format using UCSF Chimera 1.17.3 software. The molecule was optimised using the Conjugate Gradients method in Avogadro 1.1.0 software and then converted to PDBQT format using AutoDock Tools 1.5.7.

Docking procedure: Ligands were docked into the active site of the protein using Autodock Vina software. Discovery Studio Visualizer 4.0 software was utilised to observe the interactions between the protein and scutellarin.

Docking results evaluation: To evaluate the docking results, the co-crystallised ligand is re-docked into the target binding site after being separated from the protein. The docking process is considered reliable if the root mean square deviation (RMSD) value is less than 1.5 Å. For scutellarin, the binding affinity is assessed through

its interactions with amino acids in the active site and the interaction energy calculated by the scoring function of Autodock Vina.

2.2. Evaluation of Lipinski's rule of five criteria

We used the Lipinski's rule of five online tool (http://www.scfbio-iitd.res.in/software/drugdesign/lipinski.jsp) to evaluate the potential of scutellarin as a therapeutic drug. The chemical structure of scutellarin was downloaded from the PubChem database in SDF format and set at pH 7.0. A compound is considered "drug-like" if it meets at least 2 of the 5 criteria: molecular weight (MW), high lipophilicity (LogP), hydrogen bond donors (HBD), hydrogen bond acceptors (HBA1), and molar refractivity (MR) [13]. Once drug-like compounds are selected, further analysis of pharmacokinetic parameters, toxicity, and molecular dynamics is conducted to yield the final results.

2.3. Prediction of pharmacokinetic and toxicity parameters

The predicted results for the pharmacokinetic parameters, including absorption, distribution, metabolism, excretion, and toxicity (ADMET) of scutellarin, were evaluated using the pkCSM tool (http://biosig.unimelb.edu.au/pkcsm/prediction) with its SMILE (simplified molecular input line entry system) molecular formula. These predictions are critical for assessing the compound's potential success as a drug.

2.4. Bioavailability radar

A bioavailability radar analysis was conducted to determine the drug-likeness of scutellarin. Using the online tool SwissADME (http://www.swissadme.ch/index.php), a bioavailability radar was generated by considering six physicochemical properties: size, solubility, flexibility, polarity, lipophilicity, and saturation. The radar plot evaluates drug-likeness by depicting a pink area within which the molecule's plot must fall to be considered drug-like.

3. Results

3.1. Validation of molecular docking

Before screening compounds, it is essential to evaluate the accuracy of the docking model. After re-docking the co-crystallised ligands, the results yielded RMSD values of 0.047, 0.017, and 0.601 Å, respectively (Figs. 1A-F). All three values meet the criterion of RMSD being less than 1.5 Å, indicating that the molecular docking results for the targets are reliable.

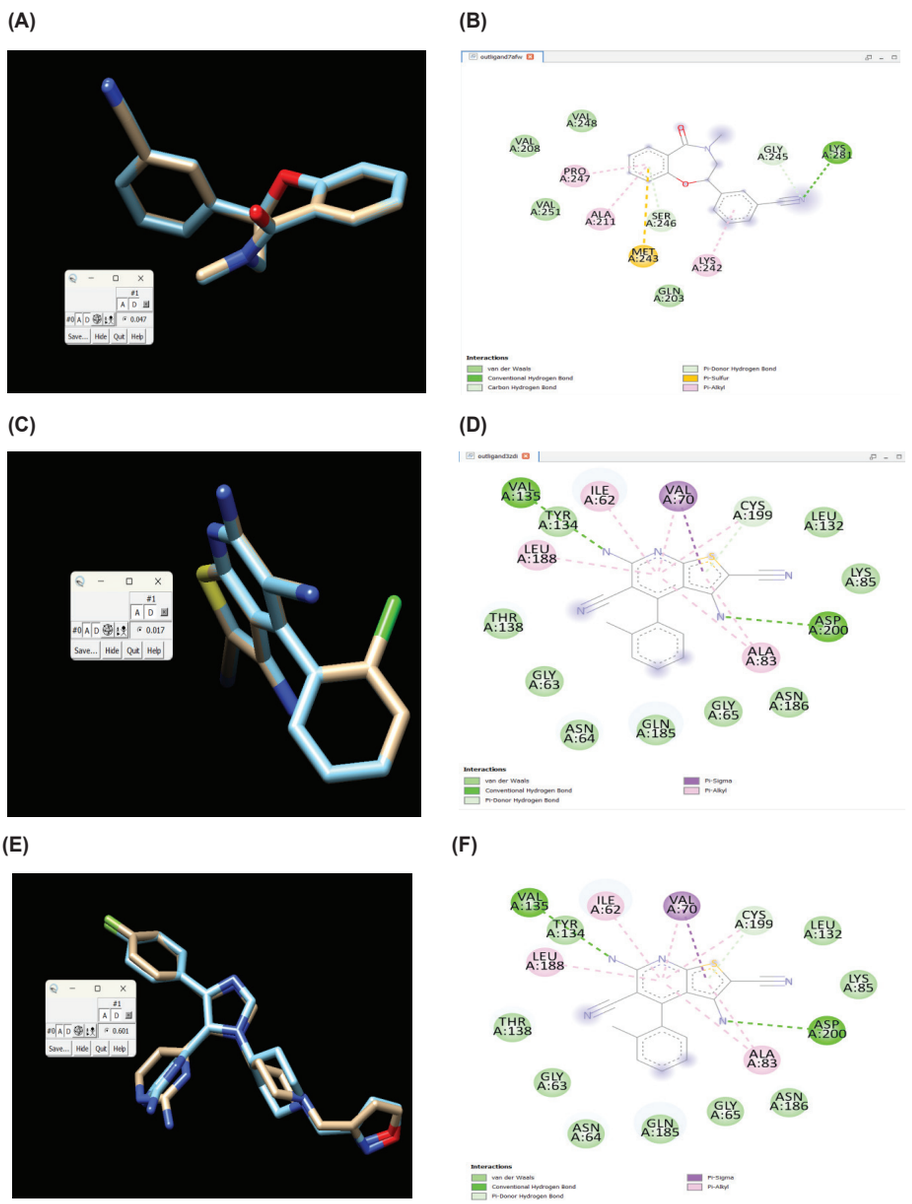

Fig. 1. (A) Root mean square deviation results of R9Q and 7AFW; (B) 2D interaction between R9Q and 7AFW; (C) Root mean square deviation results of UGJ and 3ZDI; (D) 2D interaction between UGJ and 3ZDI; (E) Root mean square deviation results of PFO and 4TN6; (F) 2D interaction between PFO and 4TN6.

3.2. Docking results of scutellarin on targets in the wnt/β-catenin pathway

Following ligand preparation, scutellarin was docked to evaluate its inhibitory activity against targets in the wnt/β-catenin pathway. Scutellarin exhibited binding energies of -6.6, -9.1, and -9.5 kcal/mol with beta-catenin-1, glycogen synthase kinase-3 beta, and casein kinase I isoform delta, respectively. In comparison, the co-crystallised ligands R9Q, UGJ, and PFO displayed binding energies of -6.3, -8.6, and -8.8 kcal/mol, respectively, for the same targets.

Binding energy (kcal/mol) correlates with the binding affinity of ligands or inhibitors to their respective target proteins. Generally, lower binding energy signifies higher affinity between ligand and receptor. These results demonstrate that scutellarin exhibits strong inhibitory potential against the three targets-beta-catenin-1, glycogen synthase kinase-3 beta, and casein kinase I isoform delta-outperforming the co-crystallised ligands for each target.

3.3. Results of Lipinski's rule of five

A compound is considered "drug-like" if it satisfies at least two of the five criteria of Lipinski's rule of five: (1) molecular weight <500 Da; (2) high lipophilicity (logP <5); (3) no more than 5 hydrogen bond donors; (4) no more than 10 hydrogen bond acceptors; (5) molecular polar surface area within 40-130 Å [13]. Scutellarin's compliance with these criteria is summarised in Table 2. Furthermore, its pharmacokinetics and toxicity (ADMET) properties were also evaluated.

Table 2. Results of Lipinski's rule of five.

Compound	Molecular weight	Donors of hydrogen bonding	Acceptors of hydrogen bonding	logP	Molar refractivity	Drug-likeness
Scutellarin	462	7	12	-0.3094	105.76	Yes

The results in Table 2 indicate that scutellarin satisfies all five criteria, suggesting it possesses highly drug-like properties. Consequently, further evaluation of its pharmacokinetic characteristics, including absorption, distribution, metabolism, excretion, and toxicity, was conducted.

3.4. Absorption, distribution, metabolism, excretion, and toxicity prediction

The predicted results for ADMET, including absorption, distribution, metabolism, excretion, and toxicity, are presented in Table 3.

Table 3. Predicted pharmacokinetic and toxicity results.

Properties		Results
Absorption	Water solubility (log mol/l)	-2.795
	CaCo2 permeability ($logP_{app}$ in 10^{-6} cm/s)	-0.911
	Intestinal absorption human (%)	13.836
Distribution	VDss (log L/kg)	0.904
	BBB permeability (logBB)	-1.502
Metabolism	CYP2D6 substrate	No
	CYP3A4 substrate	No
	CYP2D6 inhibitor	No
	CYP3A4 inhibitor	No
Excretion	Total clearance (log ml/min/kg)	0.481
	OCT2 renal substrate	No
Toxicity	AMES toxicity	No
	Hepatotoxicity	No
	Dermal sensitisation	No

The absorption capacity of compounds is evaluated based on three key characteristics: water solubility, permeability through Caco2 membranes, and the drug absorption rate in the intestine. A compound is considered to have good permeability if its Caco2 membrane permeability exceeds 0.9 [14]. The results in Table 3 indicate that scutellarin has a permeability value below 0.9, demonstrating low membrane permeability. Scutellarin also exhibits poor water solubility, with a molar concentration of approximately 10^{-3} mol/l, and low intestinal absorption of around 14%.

In terms of distribution, compounds are deemed well-distributed to tissues if the log VDss value exceeds 0.45 and poorly distributed if it is less than -0.15 [14]. A higher VDss value indicates greater drug distribution into tissues relative to plasma. Scutellarin has a log VDss of 0.904, suggesting good tissue distribution. The ability of a drug to cross the blood-brain barrier (BBB) is crucial for reducing toxicity, minimising side effects, or enhancing efficacy for drugs with central nervous system activity. Compounds are capable of crossing the BBB if their logBBB value exceeds 0.3. Scutellarin, however, does not cross the BBB, as indicated by its logBBB value of -1.502.

Regarding metabolism, the cytochrome P450 system plays a pivotal role in liver drug metabolism. P450 inhibitors can significantly alter drug pharmacokinetics. The two main polymorphic forms of cytochrome P450 involved in drug metabolism are CYP2D6 and CYP3A4. Scutellarin is neither a substrate nor an inhibitor of CYP2D6 or CYP3A4, suggesting it does not interfere with these metabolic pathways.

In terms of elimination, scutellarin's pharmacokinetics were evaluated based on total clearance and its potential as an OCT2 substrate in the kidneys. Organic Cation Transporter 2 (OCT2) is a renal transporter that plays a key role in the processing and elimination of both drugs and endogenous compounds [14]. Scutellarin is not an OCT2 substrate and is excreted through the kidneys with a total clearance rate of 0.481 (log ml/min/kg).

With respect to toxicity, scutellarin is predicted to be non-toxic based on the AMES test, non-hepatotoxic, and non-sensitising to the skin.

Based on the predicted ADMET results, scutellarin exhibits the following pharmacokinetic properties: poor absorption (due to low Caco2 membrane permeability, low water solubility, and poor intestinal absorption), good tissue distribution (VDss = 0.904>0.45), inability to cross the blood-brain barrier, and neither substrate nor inhibitor activity for major liver enzymes (CYP2D6 and CYP3A4), indicating no adverse impact on liver efficacy or toxicity. It is excreted through the kidneys with a clearance rate of 0.481 (log ml/min/kg) and exhibits no toxicity in the liver, skin, or the AMES test.

However, further in-depth studies are required to optimise and address the compound's limitations, aiming to position scutellarin as a promising candidate for the development of new cancer treatments.

3.5. Bioavailability radar

Based on efficacy and toxicity, poor bioavailability is often considered a major cause of drug development failure, and oral administration is the most common route of administration. Therefore, the oral bioavailability radar chart uses six physicochemical properties - lipophilicity, size, polarity, insolubility, flexibility, and saturation - to assess oral bioavailability.

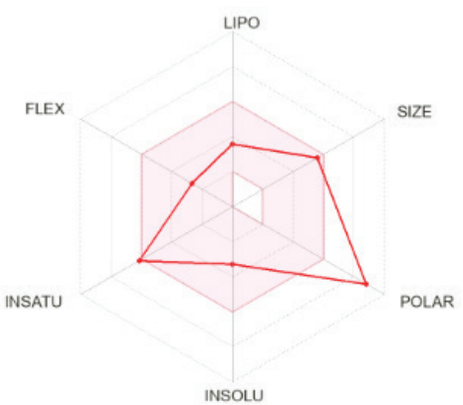

Fig. 2. Bioavailability radar of scutellarin.

Table 4. Bioavailability radar chart of scutellarin.

Parameter	Reference range	Scutellarin
Lipophilicity (XLOGP3)	0.7-5.0	0.75
Size (MW)	150-500 (g/mol)	462.36
Polarity (TPSA)	20-130 Å	207.35
Saturation (Fraction Csp3)	0.25-1	0.24
Solubility (LogS (ESOL))	-6-0	-3.27
Flexibility (Num.rotatable bonds)	≤9	4

Scutellarin exhibits optimal lipophilicity, size, solubility, and flexibility. However, it demonstrates high polarity and slightly lower saturation compared to the reference range (Fig. 2 and Table 4).

4. Discussion

The incidence of colon cancer is rising, with a high associated mortality rate. In addition to conventional treatments such as surgery, radiotherapy, and chemotherapy, targeted therapy has emerged as a promising option, particularly for patients with advanced or metastatic colon cancer. Unlike traditional chemotherapy, which typically targets all rapidly dividing cells (both cancerous and normal), targeted therapy acts specifically on certain proteins or genes in cancer cells. This specificity helps to minimise side effects while increasing treatment efficacy. The wnt/β-catenin pathway has been identified as a novel target for colon cancer therapy, as the abnormal accumulation of β-catenin is a major contributor to the disease. Consequently, this pathway presents an attractive target for drug development in colon cancer treatment.

Scutellarin, a natural flavonoid derived from various plant sources, has demonstrated significant inhibitory potential against three key targets in the wnt/β-catenin pathway: beta-catenin-1, glycogen synthase kinase-3 beta (GSK-3β), and casein kinase I isoform delta (CK1δ). Scutellarin satisfies all five criteria of Lipinski's rule of five, indicating high drug-likeness. Furthermore, it exhibits favourable pharmacokinetic properties and shows no detectable toxicity. This bioactive compound class has diverse pharmacological applications and is widely employed in the treatment of conditions such as stroke, angina, cerebral thrombosis, and coronary heart disease. Scutellarin represents a promising candidate for further research and development.

In recent years, research has highlighted scutellarin's potential therapeutic effects against tumours, inflammation, oxidative stress, viral infections, and metabolic disorders, as well as its protective effects on the kidneys [15]. Its promising role in cancer therapy has also been explored [10]. The downregulation of GSK-3, CK1, and β-catenin in the wnt/β-catenin pathway has been shown to inhibit cancer progression [16].

In this study, scutellarin demonstrated the ability to inhibit the activity of these proteins. Among the three targets, scutellarin exhibited the strongest binding affinity with CK1δ, as evidenced by the lowest binding energy and the most diverse range of chemical interactions. CK1 is involved in several cellular processes, including membrane transport regulation, cell cycle progression, vesicle trafficking, ribosome synthesis, DNA repair, signalling pathways, and circadian rhythms [17-19]. Among the CK1 isoforms, CK1δ plays a significant role in modulating canonical wnt signalling by phosphorylating lymphoid enhancer-binding factor 1 (LEF-1). This phosphorylation disrupts the β-catenin/LEF-1 complex, thereby inhibiting cell growth pathways [20].

Studies have shown that a point mutation in the C-terminal regulatory domain of CK1δ (R342H) can drive colon cancer independently of the wnt/β-catenin pathway [20]. Conversely, the downregulation of CK1δ and CK1ϵ has been associated with cell cycle arrest and apoptosis in various cancer cell lines. *In vitro* and animal studies have demonstrated that reduced CK1δ activity inhibits tumour progression, such as in breast cancer models [20]. Scutellarin interacts with CK1δ through hydrogen bonds (ASN133, LYS130, SER19), π-sigma bonds (MET82, LYS38), π-alkyl bonds (ALA36, ILE148, ILE23), and multiple van der Waals interactions, stabilising its binding at the CK1δ active site (Fig. 3A). With a binding energy of -9.5 kcal/mol, scutellarin is predicted to strongly inhibit CK1δ, potentially preventing tumour formation and cancer progression.

Scutellarin also showed a weaker binding affinity for β-catenin (binding energy ΔG=-6.5 kcal/mol). This interaction involves van der Waals forces, π-sigma interactions with MET243, and hydrophobic interactions with LYS242 and THR205, stabilising its binding at the β-catenin active site (Fig. 3B).

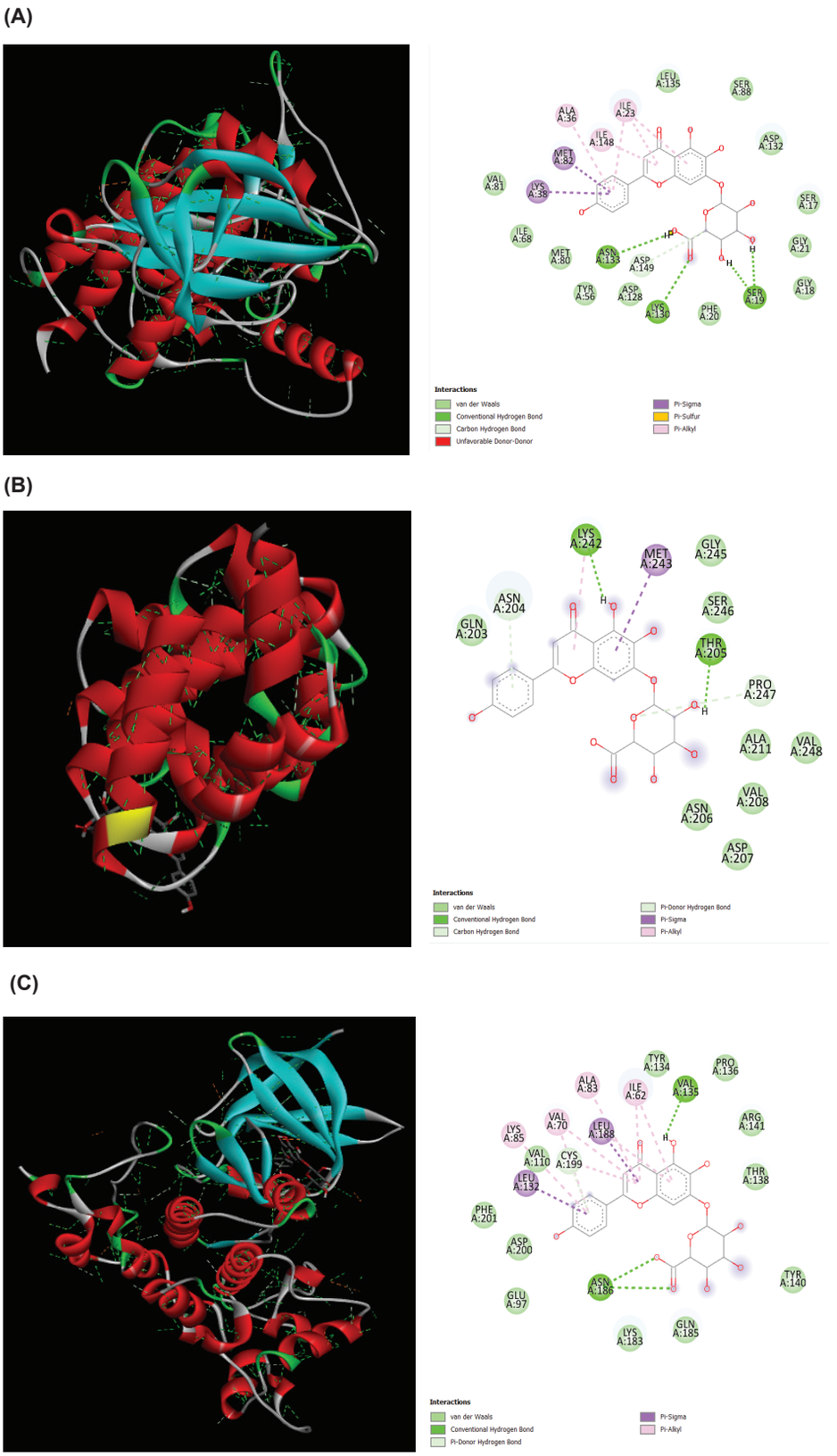

Fig. 3. (A) Interaction of scutellarin with casein kinase I isoform delta (PDB ID: 4TN6); (B) Interaction of scutellarin with beta-catenin-1 (PDB ID: 7AFW); (C) Interaction of scutellarin with glycogen synthase kinase-3 beta (PDB ID: 3ZDI).

GSK-3β, another target, plays a role in energy metabolism, neuronal development, and body pattern formation [21]. It is a key component of the wnt signalling pathway, where its phosphorylation of β-catenin inhibits signalling in the absence of wnt ligands [22]. Dysfunctions in GSK-3β have been demonstrated to be associated with the pathogenesis of various human diseases, including cancer [23]. In colorectal, ovarian, and pancreatic adenocarcinomas, GSK-3β has been reported to promote the proliferation and survival of cancer cells [24-26].

Furthermore, GSK-3β is known to be regulated by the canonical wnt pathway (WCP). In the absence of WCP stimulation, GSK-3β forms a "destruction complex" with other proteins, including adenomatous polyposis coli (APC), axin, and CK1. This complex promotes the phosphorylation and proteasomal degradation of β-catenin in the absence of wnt ligands. Upon wnt pathway activation, the destruction complex is disrupted, leading to GSK-3β inactivation, cytoplasmic accumulation of β-catenin, and subsequent nuclear translocation, where it forms a transcriptional complex with T-cell factor/lymphoid enhancer factor (TCF/LEF) [27].

Scutellarin interacts with GSK-3β through hydrophobic interactions (VAL135, ASN186), π-sigma interactions (LEU132, LEU188), π-alkyl interactions (LYS85, VAL70, ALA83, ILE62), and several van der Waals forces, stabilising its binding at the GSK-3β active site (Fig. 3C).

5. Conclusions

This study demonstrates the interaction of scutellarin with three key targets-CK1δ, GSK-3β, and β-catenin-in the wnt/β-catenin pathway, highlighting its potential in cancer therapy. Scutellarin meets Lipinski's drug-likeness criteria and exhibits favourable ADMET properties. As a natural compound, scutellarin has shown efficacy in cancer treatment across various mechanisms. However, further *in vitro* and *in vivo* studies are essential to optimise its pharmacological properties and validate its potential as a therapeutic agent for colon cancer.

CRediT author statement

Chuc Nguyen Thanh, Hoa Tong Thi Thu: Resources, Literature search, Writing; Tung Bui Thanh: Conceptualisation, Designing, Supervision, Critical reviews.

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COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

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