Original Article

Integrating network pharmacology and computational biology to propose Yiqi Sanjie formula's mechanisms in treating NSCLC: molecular docking, ADMET, and molecular dynamics simulation

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Background: Non-small cell lung cancer (NSCLC) remains a leading cause of cancer-related deaths globally. Current treatments often do not fully meet efficacy and quality of life expectations. Traditional Chinese medicine (TCM), particularly the Yiqi Sanjie formula, shows promise but lacks clear mechanistic understanding. This study addresses this gap by investigating the therapeutic effects and underlying mechanisms of Yiqi Sanjie formula in NSCLC.

Methods: We utilized network pharmacology to identify potential NSCLC drug targets of the Yiqi Sanjie formula via the Traditional Chinese Medicine Systems Pharmacology (TCMSP) database. Compounds with favorable oral bioavailability and drug-likeness scores were selected. Molecular docking was conducted using AutoDock Vina with structural data from the Protein Data Bank and PubChem. Molecular dynamics (MD) simulations were performed with Desmond Molecular Dynamics System, analyzing interactions up to 500 nanoseconds using the OPLS4 force field. ADMET predictions were executed using SwissADME and ADMETlab 2.0, assessing pharmacokinetic properties.

Results: Using network pharmacology tools, we performed Search Tool for the Retrieval of Interaction Genes/Proteins (STRING) analysis for protein-protein interaction, Kyoto Encyclopedia of Genes and Genomes (KEGG) for pathway enrichment, and gene ontology (GO) for functional enrichment, identifying crucial signaling pathways and biological processes influenced by the hit compounds bifendate, xambioona, and hederagenin. STRING analysis indicated substantial connectivity among the targets, suggesting significant interactions within the cell cycle regulation and growth factor signaling pathways as outlined in our KEGG results. The GO analysis highlighted their involvement in critical biological processes such as cell cycle control, apoptosis, and drug response. Molecular docking simulations quantified the binding efficiencies of the identified compounds with their targets—CCND1, CDK4, and EGFR—selected based on high docking scores that suggest strong potential interactions crucial for NSCLC inhibition. Subsequent MD simulations validated the stability of these complexes, supporting their potential as therapeutic interventions. Additionally, the novel identification of ADH1B as a target underscores its prospective significance in NSCLC therapy, further expanded by our comprehensive bioinformatics approach.

Conclusions: Our research demonstrates the potential of integrating network pharmacology and computational biology to elucidate the mechanisms of the Yiqi Sanjie formula in NSCLC treatment. The identified compounds could lead to novel targeted therapies, especially for patients with overexpressed targets. The discovery of ADH1B as a therapeutic target adds a new dimension to NSCLC treatment

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strategies. Further studies, both *in vitro* and *in vivo*, are needed to confirm these computational findings and advance these compounds towards clinical trials.

Keywords: Non-small cell lung cancer (NSCLC); traditional Chinese medicine (TCM); network pharmacology; molecular docking simulations

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Introduction

In recent years, primary lung cancer has become a leading cause of cancer-related deaths worldwide (1), with high incidence and mortality rates. Statistics show that approximately 350 people die from lung cancer each day, which is nearly 2.5 times the number of deaths from the second leading cause of cancer-related deaths (colorectal cancer) (2). Lung cancer can be classified into two main types based on histopathology: small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). NSCLC is the most common type of lung cancer in clinical practice, accounting for approximately 82% of all lung cancer cases (3). Patients with NSCLC have a poor prognosis, with a 5-year survival rate of only 26% (4). Although Western medicine has shown promising results in the treatment of lung cancer, it also incurs problems that undermine the benefits of treatment. These problems include drug toxicities and side effects, drug resistance, and tumor recurrence (5), which not only significantly weaken the effectiveness of anti-tumor therapies but also lead to a drastic decrease in patients' quality of life. Therefore, the combination of modern and traditional medicine has become the best choice and an inevitable trend for the future.

Yiqi Sanjie formula is widely used in the clinical practice including the treatment of lung neoplasm (6). This formulation is regularly used in the Traditional Chinese Medicine (TCM) department of Shanxi Provincial Cancer Hospital. Its combined use with Changchun Ruibin and platinum has been widely recognized for its therapeutic effect (7). However, the molecular mechanisms of its action against NSCLC are not yet fully understood.

TCM network pharmacology (8) evaluates therapeutic potential of Chinese herbal compounds by screening for the potentially affected disease-related genes, predicting their targets and potential pharmacological effects and revealing the potential association between drug-gene-disease. Previous study has shown that TCM has the characteristics of "multiple components, multiple targets, and multiple pathways" (9). Computational methods are often used to propose the interplay between TCM compounds and human disease genes by prediction their mechanism of action (10-12). In this study, we utilized network pharmacology to identify potential targets of the active compounds in Yiqi Sanjie formula. Furthermore, *in silico*

Highlight box

Key findings

- The study successfully identified three stable drug-target combinations (CCND1-bifendate, CDK4-xambioona, and EGFR-hederagenin) through molecular docking, dynamic simulation, and absorption, distribution, metabolism, excretion, and toxicity analysis, highlighting potential anti-non-small cell lung cancer (NSCLC) effects of the Yiqi Sanjie formula.
- Molecular dynamic simulation with site-directed mutagenesis further explored the structure-activity relationship between Bifendate and key residue sites, providing new insights into NSCLC targeted therapy.

What is known and what is new?

- The therapeutic efficacy of traditional Chinese medicine (TCM), specifically Yiqi Sanjie formula, in alleviating NSCLC symptoms is recognized, yet its molecular mechanisms of action were not fully understood.
- This manuscript adds comprehensive computational insights into the potential mechanisms of Yiqi Sanjie formula in NSCLC treatment, identifying specific drug-target interactions and proposing new avenues for targeted therapy development.

What is the implication, and what should change now?

- The findings suggest a significant step forward in integrating TCM with modern computational biology approaches to unveil novel therapeutic targets and strategies for NSCLC treatment.
- These insights advocate for a shift towards more personalized and mechanistic-based approaches in the development of TCM therapies for cancer, emphasizing the need for further experimental validation and clinical trials to confirm these computational predictions and their therapeutic potential.

methods such as molecular docking, molecular dynamics (MD) simulations, absorption, distribution, metabolism, excretion, toxicity (ADMET) and chemical frequent hitter (ChemFH) predictive tools were employed to identify key compounds with the therapeutic potential. We present this article in accordance with the MDAR reporting checklist (available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-972/rc).

Methods

Compounds retrieval and genes collection

The list of active ingredients present in the Yiqi Sanjie formula was systematically identified using the Traditional Chinese Medicine Systems Pharmacology (TCMSP) database (13). This database employs a hybrid approach, combining quantitative computational predictions with qualitative historical data on herbal components, to screen for compounds with significant pharmacological potential. For this study, compounds, listed in Supplementary l, were filtered based on a quantitative threshold for oral bioavailability (OB) ≥30% and drug-likeness (DL) ≥0.18, ensuring that only bioactive constituents with substantial potential for effective drug development were considered (14).

The GeneCards (https://www.genecards.org/) (15) and Online Mendelian Inheritance in Man (OMIM; https://omim.org/) (16) databases were searched with a relevance score of at least 20 to identify potential targets for NSCLC. The genes of targets associated with Yiqi Sanjie formula found in TCSMP and genes related to NSCLC as indicated in GeneCards were analyzed to identify overlapping targets between bioactive constituents and diseases, which allowed the prediction of the pharmacological effects of Yiqi Sanjie formula on NSCLC. Venn diagrams of the intersection were created using the network analysis software Venny 2.1.0 (https://bioinfogp.cnb.csic.es/tools/venny/), and the number of overlapping genes was computed automatically. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Protein-protein interaction (PPI) network analysis

Potential target genes were imported into the Search Tool for the Retrieval of Interaction Genes/Proteins (STRING) database (https://cn.string-db.org/) (17) to construct a PPI network, which was analyzed using Cytoscape 3.7.2 (18) to calculate the degree values of the nodes. The node size and color were set according to their degree values, whereas the edge thickness and color were set based on the combined score. The CytoNCA plugin (19) was used to calculate betweenness centrality (BC) and adjust the network layout according to its value.

Gene ontology (GO) enrichment analysis

To establish a logical framework for gene functions and annotations (20), GO enrichment analysis was employed in this study. The Database for Annotation, Visualization, and Integrated Discovery (DAVID) database (https://david.ncifcrf.gov/) (21) was utilized with the "Homo sapiens" setting for the organism and a significance threshold of $P<0.05$ (22). Changes in genetic functions were observed in terms of molecular function (MF) and biological processes (BP), which respectively refer to genes that regulate molecular activity and biological procedures achieved through genetic programs (23). Additionally, the potential of Yiqi Sanjie formula for affecting the genetic function related to treating NSCLC was demonstrated by calculating the number of genes involved in each GO function.

Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis

The KEGG analysis could provide information on pathways of NSCLC and the pharmacological effects of Yiqi Sanjie formula. DAVID database (https://david.ncifcrf.gov/) was also used for KEGG enrichment (24) analysis and to merge KEGG pathways to construct a functional and efficient pathway network (25). The enrichment was performed in the organism setting to "Homo sapiens", with a threshold value of $P<0.05$.

Molecular docking

Key protein targets critical to NSCLC pathogenesis, such as CCND1, CDK4, and EGFR, were selected for molecular docking based on their roles in cell cycle regulation and growth factor signaling. These targets were identified through integrated network pharmacology analyses, utilizing STRING for PPIs, KEGG for pathway insights, and GO for functional importance. The three-dimensional structures of proteins and corresponding natural compounds were sourced from UniProt (https://www.uniprot.org/) (26) and Pubchem (https://pubchem.ncbi.nlm.nih.gov/) (27), respectively. AutoDock Tools 1.5.2 (28). facilitated structure

processing, which included the removal of natural ligands from the protein structures to prepare them for docking with the identified compounds from the Yiqi Sanjie formula. The GetBox Plugin.py (https://github.com/MengwuXiao/GetBox-PyMOLPlugin/blob/master/GetBox%20Plugin.py) calculated docking box parameters, ensuring targeted and efficient docking simulations. Docking was executed with AutoDock Vina 1.2.3 (29) using default settings (30), and the results were quantitatively evaluated and visually represented in a heat map. The most promising docking poses were visualized using PyMol 2.5.5 (https://pymol.org/2/) (31) and PLIP (32). Detailed information about key targets, PDB IDs, and docking box parameters can be found in Supplementary 2, providing a comprehensive resource for further verification and replication of the docking simulations. This systematic approach underscores a focused exploration of potential therapeutic interventions targeting crucial molecular interactions in NSCLC.

MD simulations

MD simulations were conducted using Desmond 2022-4 (https://www.deshawresearch.com, academic license) (33) to investigate protein-ligand interactions. Protein preparation wizard tool was used to prepare proteins for simulation by adding hydrogen atoms and setting protonation states of ionizable groups. The molecular systems for simulations were constructed using the System Builder module by assigning OPLS4 force field (34) parameters to the protein and ligand. The protein-ligand complexes was solvated into a cubic box with the size 10 Å larger that the protein-ligand complexes in all directions. The appropriate number of water molecules as well as Na^+ and Cl^- ions were added to neutralize the charges of systems. The water molecules were represented using TIP3P model. The simulation protocol included energy minimization, NVT simulation (constant temperature, constant volume) followed by NPT (constant temperature, constant pressure) simulation to equilibrate system, each for 50,000 time steps of 1 ps. A 500 ns production run MD simulation was then performed for each complex at 300 K and 1.0 atm, with energy and coordinate saving every 50 ps. The resulting trajectories were analysed using Maestro software (Schrödinger Release 2024-2: Maestro, Schrödinger, LLC, New York, NY, 2024).

ADME property assessment and lead filtering

Pharmacokinetic properties of compounds were evaluated using SwissADME (35) and ADMETlab 2.0 (36), which assessed absorption, distribution, metabolism, and excretion (ADME). The synthetic accessibility of compounds was determined using a graph attention-based assessment (GASA) tool (37). To filter out aggregators from potential lead molecules, ChemAGG (38) was utilized. In addition, ChemFluo (39) was used to filter out compounds that are known to interfere assays, including blue/green fluorescent compounds, aggregates, Fluc inhibitors, chaotic compounds, chemoreactive compounds, and other detection interference compounds.

Results

Identification of potential Yiqi Sanjie formula targets in NSCLC

The identification and analysis of potential therapeutic targets is a crucial step in drug discovery (40). In this study, the detailed analyses revealed 225 potential targets of the Yiqi Sanjie formula 225 and 495 NSCLC-related targets with 25 common targets (*Figure 1A*), such as CCND1 (PDB ID: 2w96), CDK4 (PDB ID: 7sj3), EGFR (PDB ID: 1m14) and MET (PDB ID: 1r0p) (*Figure 1B*).

Interestingly, in the PPI network of 25 target genes, ADH1B was found isolated without edges that could indicate possible PPI with other targets in the set (*Figure 1B*). ADH1B encodes a protein belonging to the alcohol dehydrogenase family, which plays a crucial role in metabolizing various substrates including ethanol, retinol, and other alcohols, crucial for detoxifying lipid peroxides and aldehydes. This protein is composed of multiple isozymes formed by homologous α, β, and γ subunits, highly active in ethanol oxidation (41). Given the link between ethanol metabolism and increased risk of cancer development, ADH1B's activity in ethanol catabolism may influence carcinogenic processes, potentially impacting NSCLC development (42). Recent findings underscore the utility of ADH1B expression levels as prognostic biomarkers in NSCLC, suggesting that higher expression correlates with more favorable outcomes. This correlation invites hypotheses that ADH1B's metabolic roles might affect tumor microenvironment or influence tumor progression pathways in NSCLC (43). Further research is necessary to elucidate the specific mechanisms by which ADH1B impacts NSCLC pathology and to explore the potential of targeting ADH1B as a therapeutic approach. Such study could reveal novel strategies for improving NSCLC prognosis and

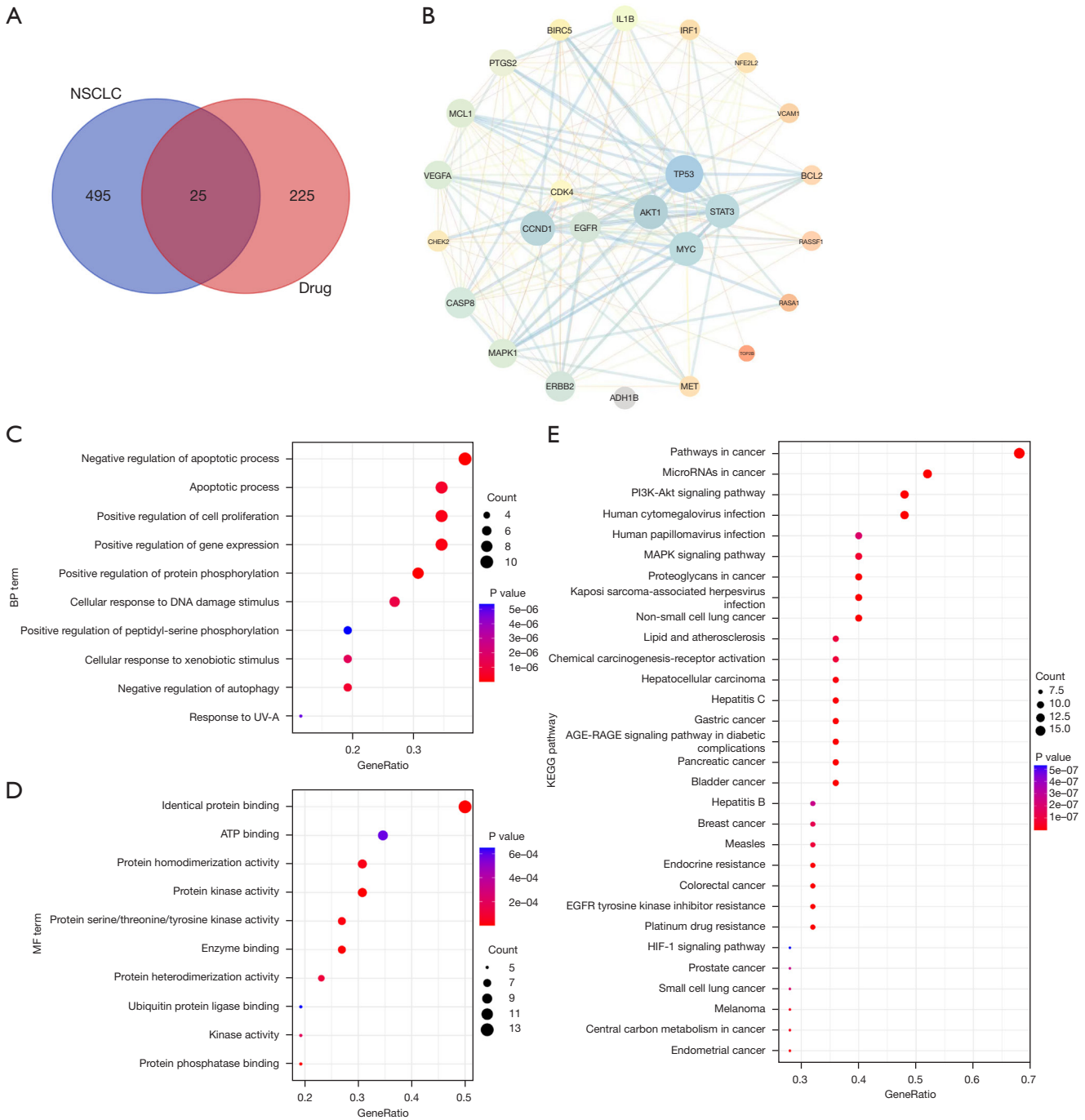

Figure 1 Network pharmacological analysis. (A) Venn diagram identifies intersection targets of drug and disease. (B) Protein interaction network diagram. (C) Biological processes enrichment analysis bubble chart of the top ten in GO. (D) Molecular functional enrichment analysis bubble chart of the top ten in GO. (E) Top 20 enriched KEGG terms bubble chart. NSCLC, non-small cell lung cancer; GO, gene ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes. ATP, adenosine triphosphate; UV-A, ultraviolet A; BP, biological processes; MF, molecular function; MAPK, mitogen-activated protein kinase; AGE, advanced glycation end-products; EGFR, epidermal growth factor receptor; HIF-1, hypoxia-inducible factor 1.

treatment, affirming the importance of ADH1B not only in metabolic processes but also in cancer biology (44).

GO analysis of potential therapeutic targets in NSCLC

To gain insight into the potential biological functions of NSCLC-related targets, GO enrichment analysis and KEGG pathway analysis were performed. Results from the top ten items of BP and MF of GO analysis showed that the 25 overlapping targets were mainly involved in signal transduction, transcriptional regulation, and apoptosis, as shown in *Figure 1C*,*1D*.

Signal transduction, transcriptional regulation, and apoptosis play important roles in the development and progression of NSCLC (45). Aberrant signaling pathways can lead to abnormal proliferation, invasion, and metastasis of tumor cells (46), whereas alterations in transcription factors and their regulatory networks can result in aberrant gene expression (47). Apoptosis is a crucial self-protective mechanism that can eliminate abnormal cells (48). Unfortunately, the exact relationship between these three MFs and NSCLC is not fully established and requires further investigation. However, previous study has shown that PPIs and molecular structural changes significantly impact tumor cell proliferation, metastasis, and apoptosis (49). Further research could reveal the specific mechanisms by which these MFs contribute to NSCLC development, providing a new theoretical basis for the development of novel targeted therapies.

In KEGG analysis, as shown in *Figure 1E*, the top 20 pathways were ranked based on the P value, and the results revealed that the targets were mainly involved in the PI3K-Akt signaling pathway, PAPK signaling pathway, AGE-RAGE signaling pathway in diabetic complications, EGFR tyrosine kinase inhibitor resistance, and HIF-1 signaling pathway. These pathways are involved in critical cellular processes such as cell growth, survival, apoptosis, and metabolism, and their dysregulation can promote NSCLC pathogenesis. Targeting these pathways has been proposed as a promising strategy for the treatment of NSCLC. For example, inhibitors of the PI3K-Akt pathway have shown potential for use as anticancer agents in NSCLC treatment (50), whereas HIF-1 inhibitors have been shown to enhance the efficacy of chemotherapy and radiotherapy in NSCLC (51). Therefore, understanding the complex interplay between these pathways and NSCLC may provide new insights into the development of targeted therapies for this disease.

Molecular docking Yiqi Sanjie formula compounds against selected NSCLC target

Molecular docking plays a crucial role in the discovery process of potential drug candidates that have an affinity to drug targets. In this study, molecular docking was used to predict the potential binding affinities between Yiqi Sanjie formula's active ingredients and 20 selected targets. To reduce computational costs while still enabling the convenient plotting of heatmaps, 20 targets were chosen instead of 25. This number strikes a balance between computational feasibility and sufficient coverage of relevant biological pathways. After excluding targets with single-segment α-helix structures, 18 potential targets were identified for further analysis. The top five targets for which ligands had the highest affinity were ADH1B, CCND1, CDK4, EGFR, and PTGS2, as shown in *Figure 2A*.

To better understand the interactions between the active ingredients and targets, the five complexes were visualized, as shown in *Figure 2B* and Table S1. It was found that hederagenin mainly interacted with EGFR through hydrophobic interactions, whereas xambioona interacted with CDK4 only through hydrogen bonds, forming a relatively simple set of intermolecular interactions. The remaining three complexes showed a more balanced interaction between the number of hydrogen bonds and hydrophobic interactions. Notably, a salt bridge was identified in the CCND1-bifendate complex, indicating potentially stronger binding stability.

MD simulation of top-scoring ligand-target complexes

Furthermore, MD simulations with the production run of 500 ns were performed for the most favorable complexes identified from the previous docking simulations, to assess the binding stability and duration. Stable and long-lasting binding would indicate the stability of the complexes and indirectly suggest the inhibitory potential of the small molecules (52). Root-mean-square deviation (RMSD) analysis, a critical tool for evaluating the stability of drug-target complex MD simulations, was performed on all five complexes. As shown in *Figure 3*, the ligand fit on protein RMSD of EGFR-hederagenin remained stable throughout the simulation, indicating a relatively stable binding between the two. However, ADH1B-Salvigenin, CCND1-bifendate, CDK4-xambioona, and PTGS2-xambioona showed varying degrees of fluctuations in their ligand fit on protein RMSD.

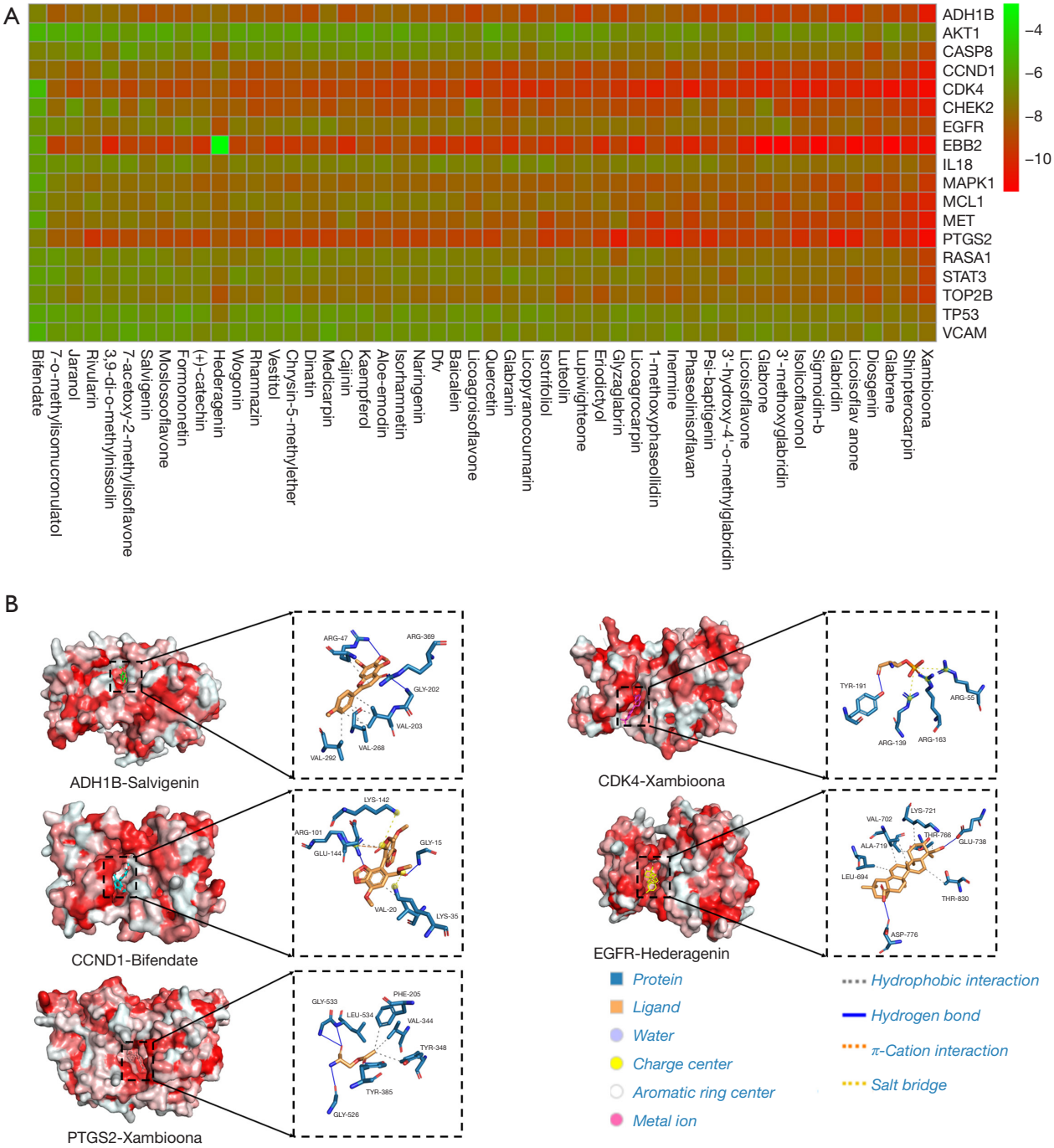

Figure 2 Results of molecular docking predictions using AutoDock Vina. (A) Docking score heatmap of all complexes. (B) Most favourable poses of five top scoring compound-target compounds.

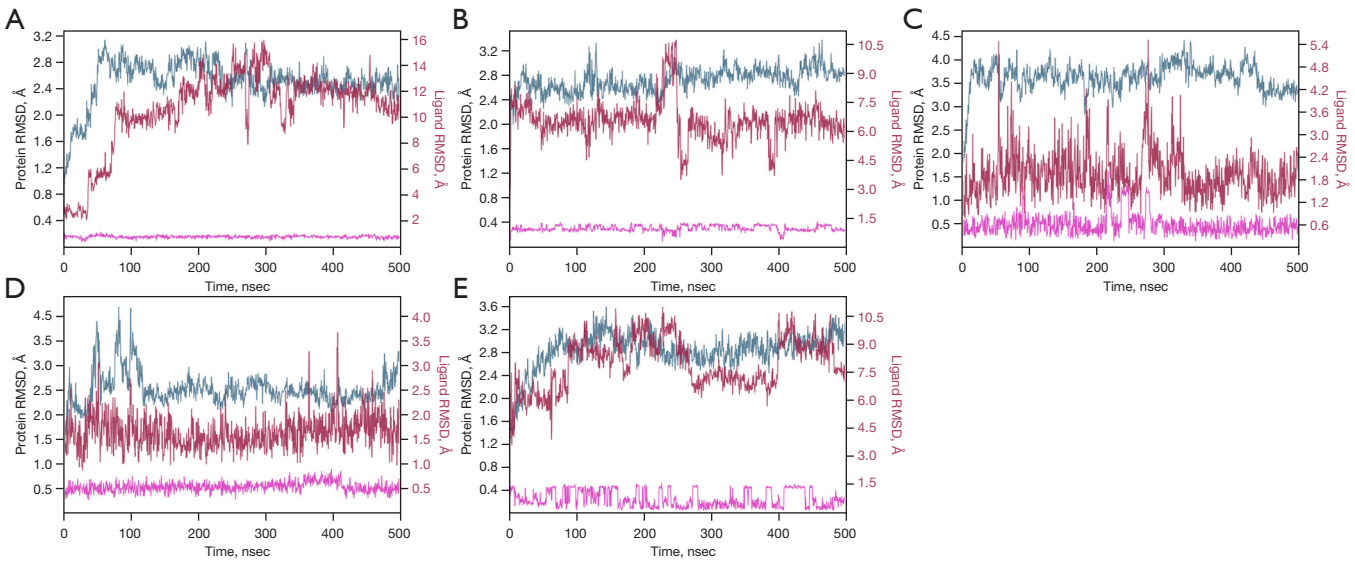

Figure 3 Dynamics of RMSD of ligand and fit on protein during 500 ns of simulation. (A) ADH1B-salvigenin. (B) CCND1-bifendate. (C) CDK4-xambioona. (D) EGFR-hederagenin. (E) PTGS2-xambioona. RMSD, root-mean-square deviation.

The RMSD analysis revealed that ADH1B-Salvigenin and PTGS2-xambioona displayed fluctuations beyond an acceptable range, indicating mobility of the ligand within the binding site and thus poor stability of these drug-target complexes. Moreover, as the KEGG analysis revealed ADH1B and PTGS2 were not involved in the relevant pathways studied, these two proteins were not further analyzed. However, CCND1-bifendate and CDK4-xambioona exhibited relatively stable protein-ligand complexes throughout the latter half of the simulation, as evidenced by smaller fluctuations in their RMSD and reaching stable plateaus.

As a new plateau was observed in the last 100 ns of the CCND1-bifendate simulation, the simulation was extended to 600 ns and there were no further significant changes in the RMSD (Figure S1). Additionally, stable hydrogen bonds were maintained between bifendate and CCND1 Ala-16 and Tyr-17 throughout the simulation, indicating that the conformational changes did not greatly affect the binding between them. The main difference between the 200–400 ns and 400–600 ns timeframes was the presence or absence of interactions with Lys-35 and Asp-158. As the simulation time was extended, the proportion of bifendate's interactions with Lys-35 and Asp-158 gradually decreased to below 30% at the 400 ns time point. Thus, to improve the binding stability of bifendate derivatives to CCND1, future efforts could focus on enhancing interactions with these two residues. Further targeted mutations are detailed in Figure S2, and the results show that all four loci mutated to Gly have different degrees of effect on bifendate binding, with D158G and A16G having the most significant effect.

Overall, three Yiqi Sanjie components (bifendate, xambioona and hederagenin) in complex with their respective targets (CCND1, CDK4 and EGFR) were considered for further analysis.

Subsequently, MD simulations were performed on the three selected targets without ligands. *Figure 4* shows the RMSD changes of the targets before and after ligand binding. The results indicated that the protein conformations were more stable after ligand binding than in the unbound state, as evidenced by the convergence of the RMSD changes to varying degrees. Therefore, it can be inferred that the three selected compounds enhanced the stability of the bound proteins to some extent.

Residue mobility pre- and post-ligand delivery analysis

Assessing the dynamic behavior of ligand-target complexes was crucial to understanding how compounds interacted with their targets and enhanced stability. Therefore, we evaluated the root-mean-square fluctuation (RMSF) changes of the targets before and after ligand binding during MD simulations. Our findings, as shown in *Figure 5*, revealed that the compounds interacted differently with their respective targets and dynamically adjusted the RMSF of specific peptide segments.

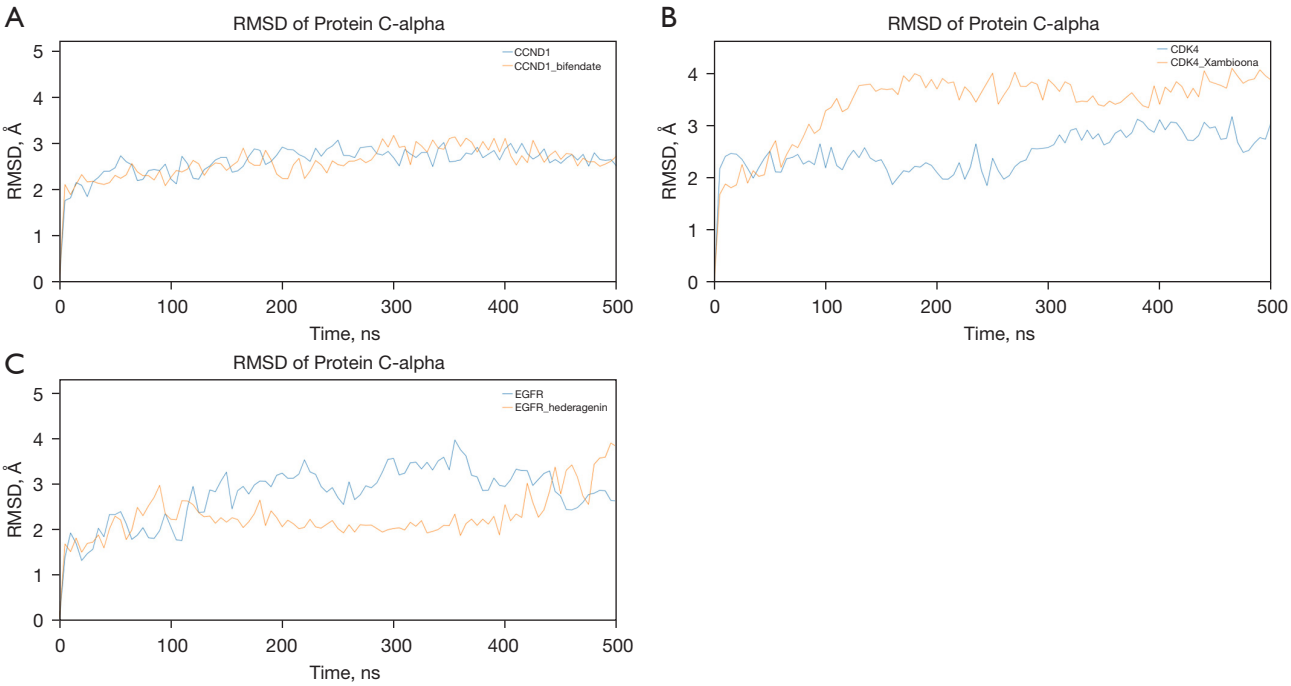

Figure 4 RMSD analysis of pre- and post-drug delivery. (A) CCND1, (B) CDK4, (C) EGFR. RMSD, root-mean-square deviation.

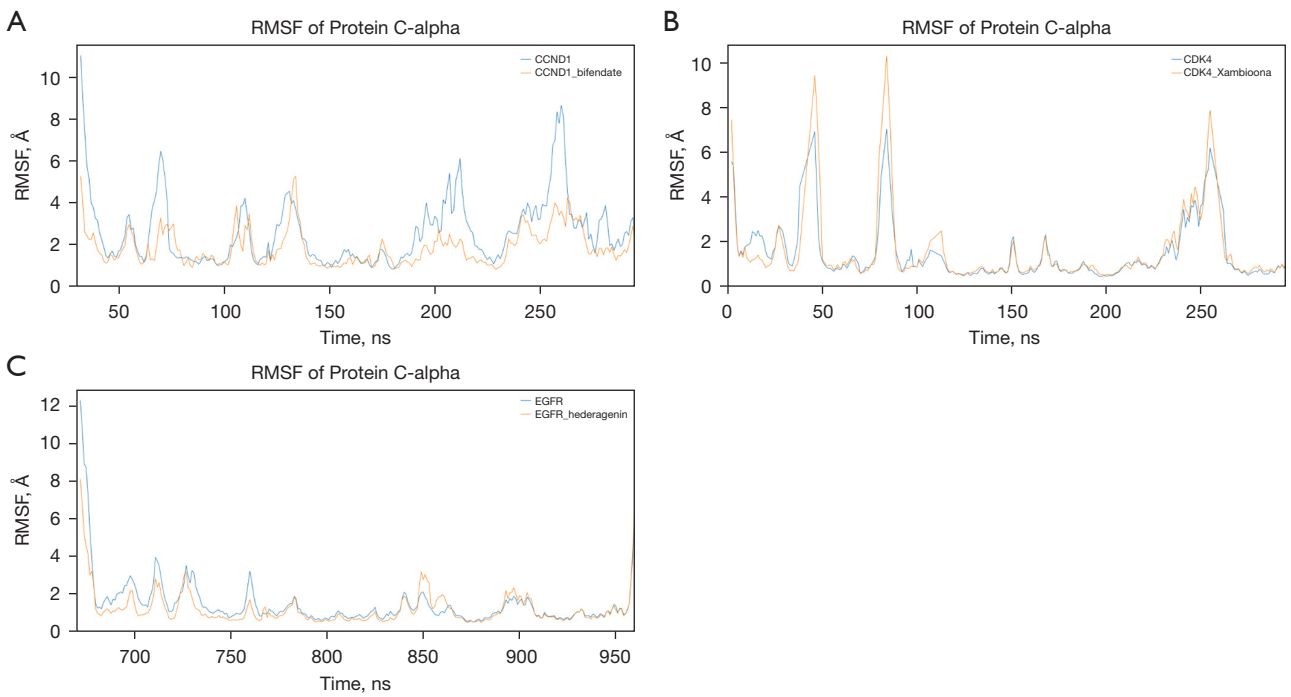

Figure 5 Flexibility of three proteins without and with natural product present in the binding site. RMSF indicates the protein flexibility using root-mean-square fluctuation for each residues. (A) CCND1, (B) CDK4, (C) EGFR. RMSF, root mean square fluctuation; EGFR, epidermal growth factor receptor.

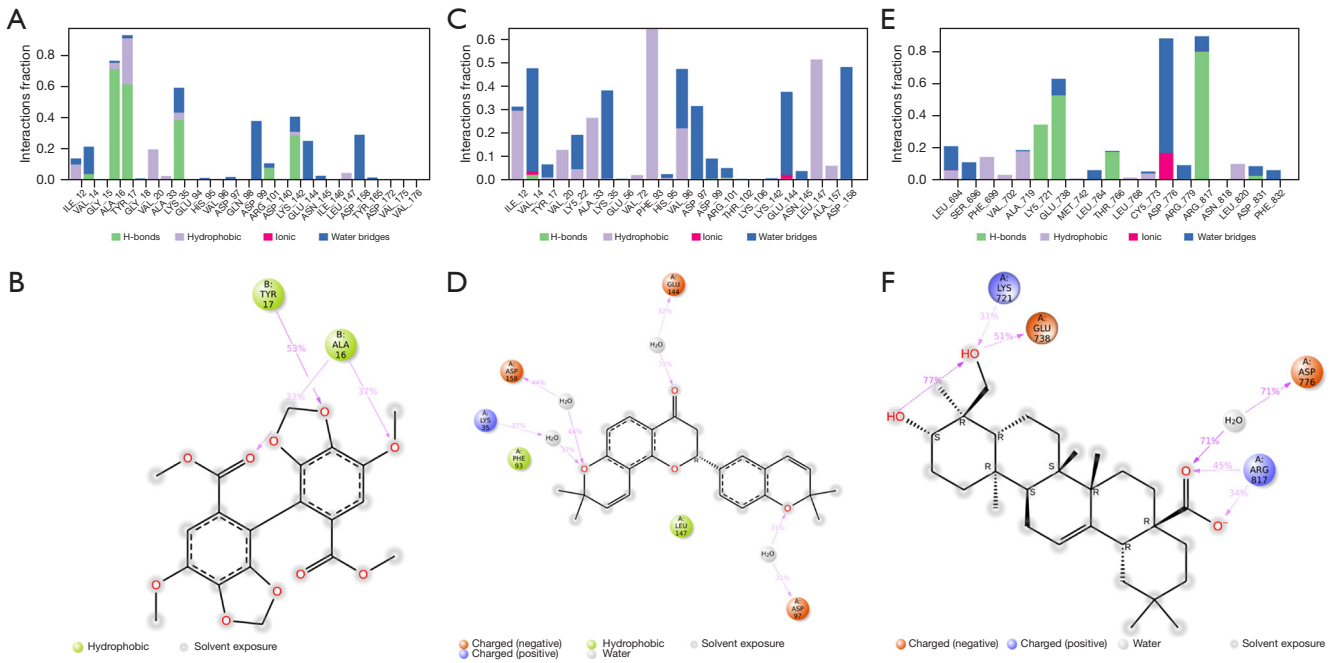

Figure 6 Contact analysis. The bar graph shows the types of interactions made by amino acid residues in the binding pocket with the ligand, and how long these interactions were maintained during the simulation. The interactions, categorized into four types: Hydrogen Bonds, Hydrophobic, Ionic, and Water Bridges, were color-coded in the stacked bar graph. (A,C,E) Histogram of ligand contacts with amino acid residues of target protein. (B,D,F) Schematic of ligand contacts with amino acid residues of target protein.

Bifendate binding to CCND1 upregulated the RMSF of the peptide segment 167–203, downregulated the RMSF of the peptide segments 98–123, and dynamically adjusted the RMSF of the peptide segments 9–65 and 215–238. Xambioona binding to CDK4 upregulated the RMSF of the peptide segments 78–85 and 278–281, downregulated the RMSF of the peptide segments 9–22 and 105–113, and dynamically adjusted the RMSF of the peptide segments 35–48 and 253–267 (partially upregulated, partially downregulated, and partially unmodified). Hederagenin binding to EGFR upregulated the RMSF of the regions 684–712, 728–738, and 756–764, and downregulated the RMSF of the region 848–862.

Based on the analysis presented earlier, it can be inferred that bifendate primarily targets the cyclin N-terminal of CCND1, although it remains unclear if it affects the key binding sites. Additional information, such as mutual interaction statistics, would be required for a more comprehensive analysis. Xambioona targets the protein kinase domain of CDK4, including binding sites 12–20 and Lys-35. However, the RMSF analysis did not reveal any impact on the active site Asp-140 of CDK4, suggesting a limited effect on its activity. Hederagenin targets the 688–704 region of EGFR, which is critical for dimerization, phosphorylation, and activation, as well as the protein kinase domain, including the active site Asp-837 and binding site Asp-855 (53).

Protein-ligands contact analysis of complexes

Estimation of protein interactions provides a measure of interaction power between the ligands and the target protein. These interactions can be categorized by type and summarized, as shown in the plot represented in *Figure 6*. In the case of CCND1-bifendate, as shown in *Figure 6A,6B*, the primary types of interactions were hydrogen bonds and water bridges, along with some hydrophobic interactions. Through the simulation trajectory, it was observed that bifendate maintained stable binding to the active pocket of CCND1 by forming hydrogen bonds with key residues, including Ala-16 and Tyr-17. These residues are known to be important constituents of the CCND1 pocket, which further confirms the specificity of bifendate in targeting CCND1. The 286–288 region (54-56) that produces activity or functional effects on CCND1 after mutation is located at the C-terminus, which is typically used for

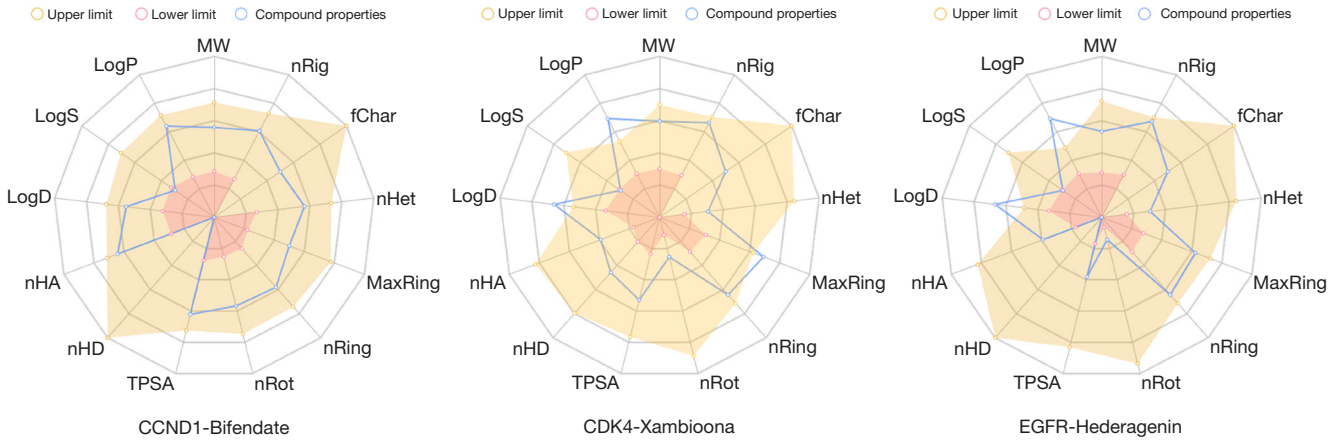

Figure 7 Physicochemical Property Radar chart. The white area is the area that exceeds the theoretical maximum value of each parameter, the yellow area is the area that conforms to the range of the theoretical value of each parameter, and the red area is the area that is lower than the theoretical minimum value of each parameter.

screening allosteric modulators. However, conventional inhibitors should still be screened based on the conventional druggable pockets. Therefore, it cannot be ruled out that bifendate may exert its inhibitory effect on CCND1 through a binding conformation in this pocket, given that multiple compound structures bound to the Ala-16 and Tyr-17 pocket have been resolved.

In the case of CDK4-xambioona, as shown in *Figure 6C,6D*, hydrophobic interactions and water bridges played a crucial role in maintaining a stable binding conformation between the compound and its target. Additionally, ionic interactions and hydrogen bonds also contributed to the interaction between the two. Water bridges were particularly essential for stabilizing the interaction between xambioona and specific residues throughout the simulation, indicating that xambioona may hinder the activity of CDK4 by blocking ATP binding to the protein.

For EGFR-hederagenin, as shown in *Figure 6E,6F*, hydrogen bonds and water bridges were the primary types of interactions, along with some hydrophobic and ionic interactions. Hederagenin interacted with specific residues through hydrogen bonds and water bridges, with nearly constant interaction with certain residues throughout the simulation. Similar to xambioona, hederagenin also impacted the adenosine triphosphate (ATP) binding site of EGFR, particularly Lys-721 (57), specifically by blocking the binding of ATP to the protein. This suggests that hederagenin may limit the activity of EGFR.

Evaluation of ADMET properties for bifendate as a drug candidate

Computational evaluation of ADMET properties is a crucial step in investigating the safety of drug candidates, as it helps to avoid drug adverse reactions and toxicity (52). This ADMET characterization is an essential part of the drug discovery process since it reduces costs and development times in clinical trials (58). The molecular properties of three compounds were calculated, such as molecular weight (MW) and volume. A radar chart was generated based on the theoretical range of drug-like compound properties, and compounds outside of this range were eliminated. The results, shown in *Figure* 7, indicate that only bifendate falls within the theoretical range of drug-like compounds. The log of the octanol/water partition coefficient (LogP) and log at physiological Ph 7.4 (LogD) of the other two compounds exceed the maximum theoretical value. Additionally, xambioona's MaxRing, the number of atoms in the biggest ring, also exceeds the theoretical range, detailed in Table S2. Therefore, only bifendate, which conforms to drug-like compound properties, was selected for ADMET analysis.

In the realm of medicinal chemistry, bifendate displays a promising potential for drug-like properties, as indicated by a high QED score of 0.672 and adherence to Lipinski and Pfizer rules (59). Furthermore, it possesses a low SA score of 2.808, suggesting relative ease of synthesis. Notably, it has not been classified as a PAINS compound.

Regarding absorption, bifendate exhibits favorable permeability in the Caco-2 intestinal permeability

model with a value of –4.606, indicating good intestinal absorption. Its MDCK permeability is relatively high at 3.7e–05, implying passive permeability. The compound does not inhibit P-glycoprotein and can serve as a substrate for it, making it suitable for both Caco-2 and MDCK cell models.

Distribution analysis reveals that bifendate has a plasma protein binding (PPB) of 73.668%, which falls below the ideal range of 90%. This suggests a higher therapeutic index, as drugs with high protein binding may be immobilized and unable to reach their intended targets. Nonetheless, the compound has a blood-brain barrier (BBB) penetration value of less than 0.3, indicating a low potential for crossing the BBB and producing pharmacological effects in the central nervous system.

Excretion analysis shows that bifendate falls within the moderate range (5–15 mL/min/kg) for clearance rate (CL), with a value of 8.376 mL/min/kg. Its $T_{1/2}$ value is 0.181, indicating that it likely has a half-life shorter than three hours. Metabolism analysis indicates that bifendate does not exhibit inhibitory effects on CYP2D6 and can undergo normal drug metabolism *in vivo*.

Toxicity analysis suggests that bifendate has a low probability of causing adverse effects, with a likelihood of less than 10% for toxic side effects such as Herg blockers, human hepatotoxicity (H-HT), AMES toxicity, and skin sensitization. Additionally, it satisfies the acute toxicity, genotoxic carcinogenicity, non-genotoxic carcinogenicity, skin sensitization, aquatic toxicity, non-biodegradable, SureChEMBL, and FAF-Drugs4 rules.

Discussion

NSCLC presents a formidable challenge in oncology (60), characterized by its heterogeneous nature and a complex molecular landscape. Despite the significant advancements in targeted therapies, the diverse genetic profiles of NSCLC tumors often result in variable responses to treatment and the development of resistance (61,62). In addressing these challenges, our study embarked on an integrative approach, combining bioinformatics, molecular docking, and dynamics simulations, to identify novel molecular targets and explore potential therapeutic strategies.

The initial phase of our study, centered on bioinformatics analysis, was pivotal in unraveling potential therapeutic targets (63). By meticulously analyzing the interactions between the components of Yiqi Sanjie formula and genes related to NSCLC, we identified several key proteins and pathways implicated in the disease. This analysis not only reinforced the known roles of proteins such as EGFR (64) and MET (65) in NSCLC but also brought to light the potential significance of ADH1B in the disease's progression (66). These insights were instrumental in steering the direction of our subsequent drug discovery and target validation efforts.

Building on this foundation, the molecular docking analysis provided significant insights into the interactions between the identified targets and the active ingredients of Yiqi Sanjie formula (67). Notably, compounds such as hederagenin and xambioona exhibited high docking scores with critical NSCLC targets, namely EGFR and CDK4, suggesting strong binding affinities. These findings are particularly promising, hinting at the potential of these compounds to disrupt key signaling pathways involved in NSCLC tumor growth and progression.

Another crucial consideration in our study is the specificity of drug targeting on the identified proteins. Specificity is essential to minimize off-target effects and enhance the therapeutic efficacy of treatments (Latest Developed Strategies to Minimize the Off-Target Effects in CRISPR-Cas-Mediated Genome Editing). The molecular docking and dynamics simulations provided valuable insights into potential binding affinities, but further experimental validation is necessary to confirm the specificity of these interactions. Particularly for compounds like hederagenin, which shows potential for targeting EGFR, it is important to thoroughly investigate its specificity against other proteins to ensure precise targeting. Similarly, for xambioona and bifendate, further studies should explore their specificity to CDK4 and CCND1, respectively. This will help ascertain their therapeutic potential while minimizing unintended effects. Thus, specificity remains an essential factor in assessing the clinical viability of these compounds, and our future work will focus on validating these findings through comprehensive *in vitro* and *in vivo* studies.

The further dimension was added to our understanding through MD simulations, which offered a more nuanced view of the stability of drug-target interactions (68). The EGFR-hederagenin complex, for instance, maintained a stable binding throughout the simulation, indicative of a robust and potentially effective therapeutic interaction (69). In contrast, the variability observed in other drug–target interactions, such as that of ADH1B-Salvigenin, highlighted the necessity for more comprehensive investigations to fully ascertain their therapeutic viability.

In our investigation, we focused on predicted interactions of natural products with three proteins involving key NSCLC targets: hederagenin with EGFR, xambioona with CDK4, and bifendate with CCND1. Notably, the inhibitory potential of hederagenin and its derivatives on EGFR is supported by existing literature (70), highlighting the validity of this pairing as a therapeutic strategy in NSCLC. This confirmation aligns well with our findings and underscores the potential of the EGFR-hederagenin interaction in NSCLC treatment. Conversely, the interactions involving xambioona and CDK4, as well as bifendate and CCND1, although promising, have yet to be extensively validated in scientific literature. Xambioona's targeting of CDK4, a key regulator of the cell cycle, suggests its potential to arrest tumor cell growth and induce apoptosis, directly addressing the dysregulation of the cell cycle observed in NSCLC (71). However, the optimization of xambioona's pharmacological properties might be necessary to fully realize its therapeutic efficacy (72). Similarly, bifendate's interaction with CCND1, coupled with its balanced ADMET profile, indicates its potential role in disrupting cell cycle progression in NSCLC, a critical aspect of the disease's pathology (73).

Conclusions

These findings collectively highlight the significant potential application value of these interactions in NSCLC therapy. While the EGFR-hederagenin pairing is already supported by existing research, the other two combinations—xambioona with CDK4 and bifendate with CCND1—represent novel and promising areas for future investigation. Their exploration could lead to groundbreaking advancements in NSCLC treatment, particularly in targeting mechanisms that are currently underexplored in existing therapeutic strategies.

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Footnote

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