Original Article

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Exploring the Role of Neoflavonoids in Inducing Cell Cycle Arrest in Cancer Cells: In Silico Study

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ABSTRACT

Background: Cancer is one of the biggest health issues in the world, and one of the characteristics of oncogenesis is the dysregulation of cell cycle progression. Cyclin-dependent kinase 4 (CDK4) is one of the key regulatory factors of G1 to S phase transition, and hence an appealing therapeutic target.
Method: This paper explores the possibility of neoflavonoids as a new CDK4 inhibitor using a combined computational method. Our initial study involved a pan-cancer analysis of CDK expression, which was conducted on several databases such as GEPIA2, TIMER 2.0 and UALCAN, and found that CDK4 was overexpressed in many malignancies, especially glioblastoma, diffuse large B-cell lymphoma and thymoma. Survival analysis also determined that high expression of CDK4 is associated with worse prognosis in a variety of cancer. The CB-Dock2 server with AutoDock Vina was used in molecular docking experiments on 26 neoflavonoid compounds against the ATP-binding site of CDK4.
Results: Our findings have revealed a number of neoflavonoids with binding affinities similar to those of known CDK4 inhibitors, with 3010628 (-10.8 kcal/mol), 102458506 (-10.5 kcal/mol) and 195328 (-10.4 kcal/mol) coming out as the most promising compounds. The interaction analysis showed in detail that these highest-ranked neoflavonoids establish important interactions with major residues of the ATP-binding pocket such as hydrogen bonds with Val96 and Asp145, and pi-pi stacking with Phe93, which resemble the binding patterns of reference inhibitors.
Conclusion: The results indicate that neoflavonoids are the potential candidates to be further developed as CDK4-targeted anticancer agents, and further experimental efforts should be done to prove their therapeutic potential.

Keywords:CDK4; Neoflavonoids; Molecular Docking; Pan-Cancer Analysis; Cancer Therapy; Drug Discovery

INTRODUCTION:

Cancer is a significant health issue worldwide, and 20 million new cases and 9.7 million deaths were reported in 2022 [1]. Although the treatment strategies such as surgery, chemotherapy, radiotherapy, and targeted therapies have improved, drug resistance and disease recurrence remain a major challenge [2, 3]. This highlights the necessity of new treatment methods that address particular molecular pathways in cancer cells.
Cyclin-dependent kinases (CDKs) are the important regulators of cell cycle progression and CDK4 plays a key role in the regulation of the G1 to S phase transition [4-6]. CDK4 dysregulation is commonly found in a variety of cancers and thus is a promising anticancer drug target. A lot of interest has been given to natural products, especially plant-based polyphenolic compounds, including flavonoids and neoflavonoids, due to their wide range of biological activities [7, 8]. These compounds have strong antioxidant, anti-inflammatory and anticancer effects and some of them have a strong kinase inhibitory effect. It is worth noting that the first CDK inhibitor to be put into clinical trials is flavopiridol, a semi-synthetic flavonoid of rohitukine [9-13].
Considering the structural resemblance of neoflavonoids and known CDK inhibitors, the proposed study will examine the possibility of neoflavonoids as CDK4 inhibitors using combined computational methods, which involve pan-cancer analysis and molecular docking experiments.

Methodology:

Pan-cancer investigation of CDKs

In this study, we utilized the Gene Expression Profiling Interactive Analysis 2 (GEPIA2) database (http://gepia2.cancer-pku.cn/#index) to compare the expression levels of CDKs 1 to 7 across various cancer types. Additionally, we analyzed the expression of these CDKs in tumor tissue versus normal tissue, preparing a heat map to visualize our findings (figure 2). GEPIA2 provides sophisticated tools that facilitate efficient analysis and interpretation of complex gene expression data, allowing researchers to explore large datasets derived from The Cancer Genome Atlas (TCGA) and Genotype-Tissue Expression (GTEx) projects [14].
In addition to GPIA 2, we used advanced tools from two other databases University of Alabama at Birmingham CANcer data analysis Portal (ULCA) (https://ualcan.path.uab.edu/) and Timer 2.0 (http://timer.cistrome.org/) to evaluate the expression of CDK 4 in different types of cancer and normal tissue (figure 3 and 4) [15, 16].
Finally, we used the GPIA 2 database to investigate the effect of CDK 4 expression on the survival of different types of cancer patients (figure 5).

Investigation of protein-protein interaction

In this research, we employed the GeneMANIA online tool to examine a set of genes pertinent to our study. GeneMANIA integrates various functional association data, such as protein interactions, co-expression, and pathway details, to forecast gene functions and pinpoint related genes. After entering CDKs list into the platform, it produced an interactive network that depicted the connections between the input genes and their predicted links to other functionally related genes (figure 6) [17].

Molecular Docking

The crystal structure of the CDK4/Cyclin D3 complex (PDB ID: 7sj3) in three dimensions (3D) was obtained in the Protein Data Bank (https://www.rcsb.org/). The 3D structure of the 26 neoflavonoid compounds (listed in Table 1) and the three known CDK4 inhibitors, namely Rohitukine (PubChem ID: 13422576), Flavopiridol (PubChem ID: 5287969), and Abemaciclib (PubChem ID: 46220502) were retrieved in SDF format in the PubChem database (https://pubchem.ncbi.nlm.nih.gov/).
The CB-Dock2 web server (https://cadd.labshare.cn/cb-dock2/index.php) was used to perform molecular docking, which uses AutoDock Vina 1.2 engine [18]. The protein and the ligand structures were subjected to the automated pre-processing pipeline of the server. In the case of the protein, it was the removal of water molecules and ions, addition of hydrogen atoms, and charge correction. The server optimized the structure of all ligands, including the addition of hydrogen and minimization of energy [18]. Moreover, the binding cavities of the protein were automatically identified by the server [18]. The docking site was the cavity that is associated with the ATP-binding site.
The calculated parameters of the CB-Dock2 server that were inputted into the docking parameters of this cavity included: the grid box was centered at coordinates $x = 17, y = -43, z = 9$ with dimensions of 25 Å × 25 Å × 25 Å; an exhaustiveness value of 20 was applied; and 5 poses were generated per ligand. The resulting poses were grouped according to Root-Mean-Square Deviation (RMSD) criteria and the pose with the best binding affinity score was chosen to be further analyzed. All the

default AutoDock Vina scoring was employed. Discovery Studio Visualizer software was used to visualize and analyze the protein-ligand interactions of the selected poses.

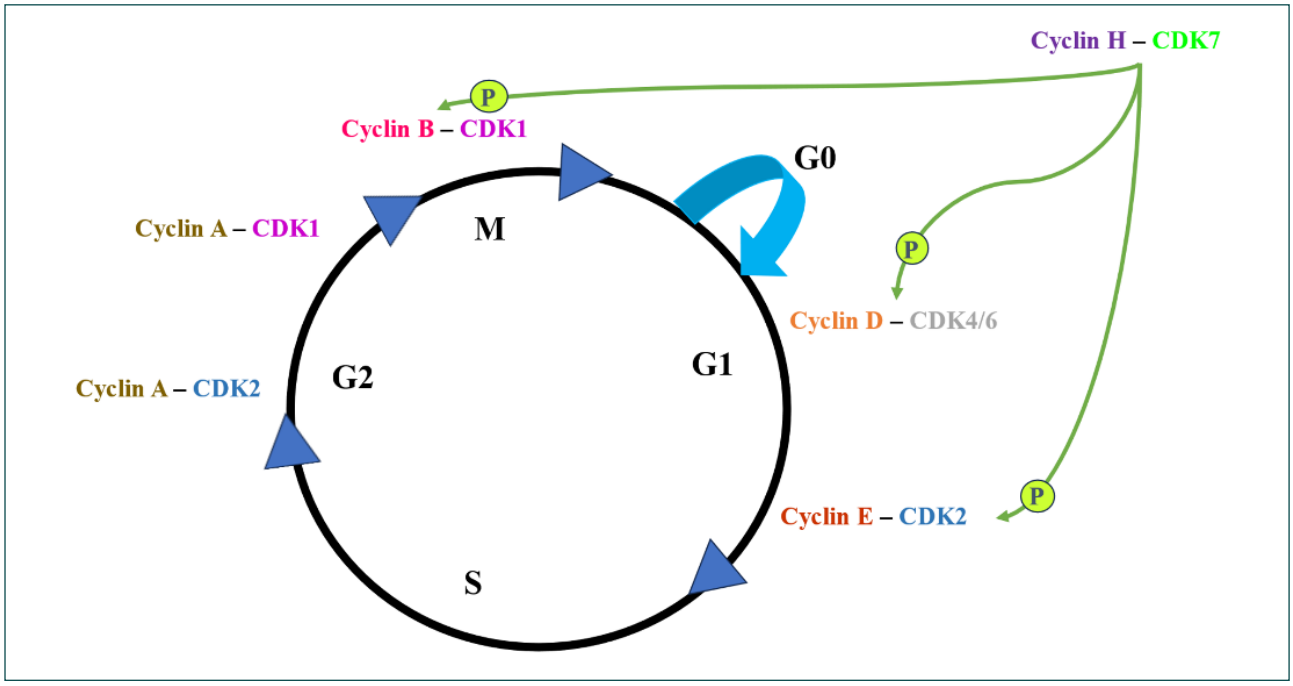

Figure 1: Khe role of different types of CDKs and cyclins in the regulation and progression of different stages of the cell cycle.

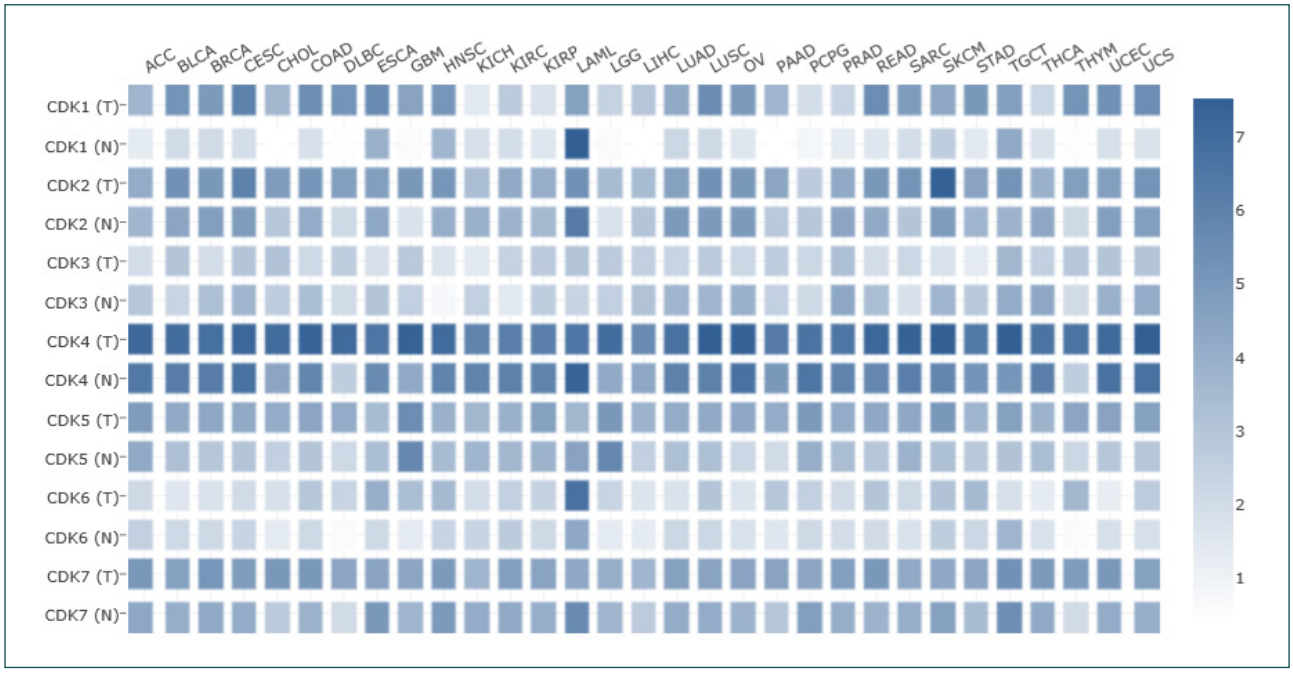

Figure 2: Heat map to investigate the expression of CDK 1 to 7 in normal and tumor tissue of d ifferent types of cancers. Tumor (T), normal (N).

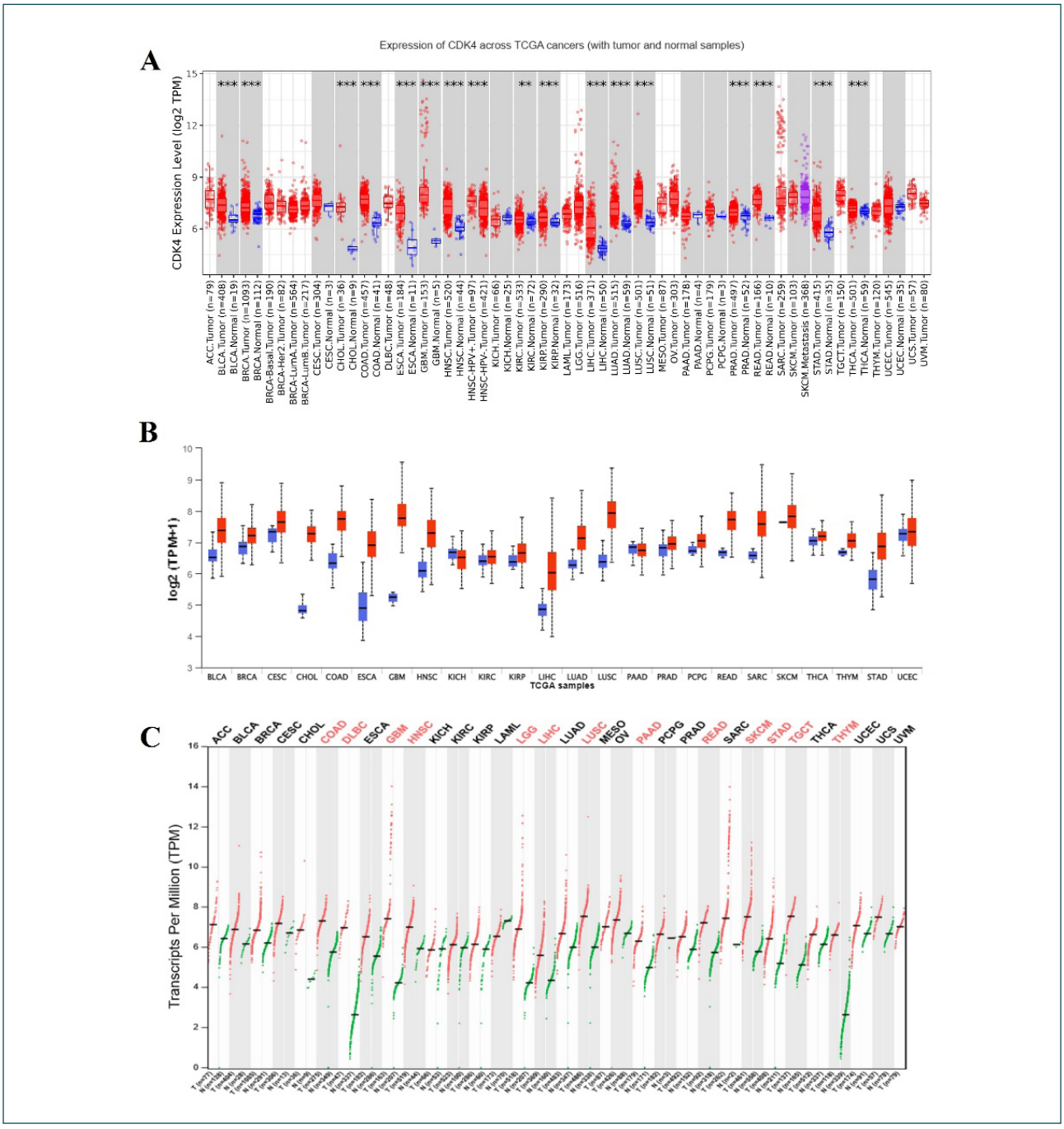

Figure 3: Hxpression of CDK4 across TCGA cancers (with tumor and normal samples); (A) Box plots of CDK 4 gene expression in the Timer 2.0 database, in this plot, the cancers for which normal tissue samples were available are in the gray column. Also, the statistical significance computed by the Wilcoxon test is annotated by the number of stars (*: p-value < 0.05; **: p-value < 0.01; ***: p-value < 0.001), the number of normal and cancerous samples is indicated below the graph; (B) Box plots of CDK 4 gene expression from the ULCAN database, the blue box corresponds to normal samples and the red box corresponds to cancer samples; (C) Dot plots of CDK 4 gene expression of GPIA 2 database, green dots correspond to normal samples and red dots correspond to cancer samples.

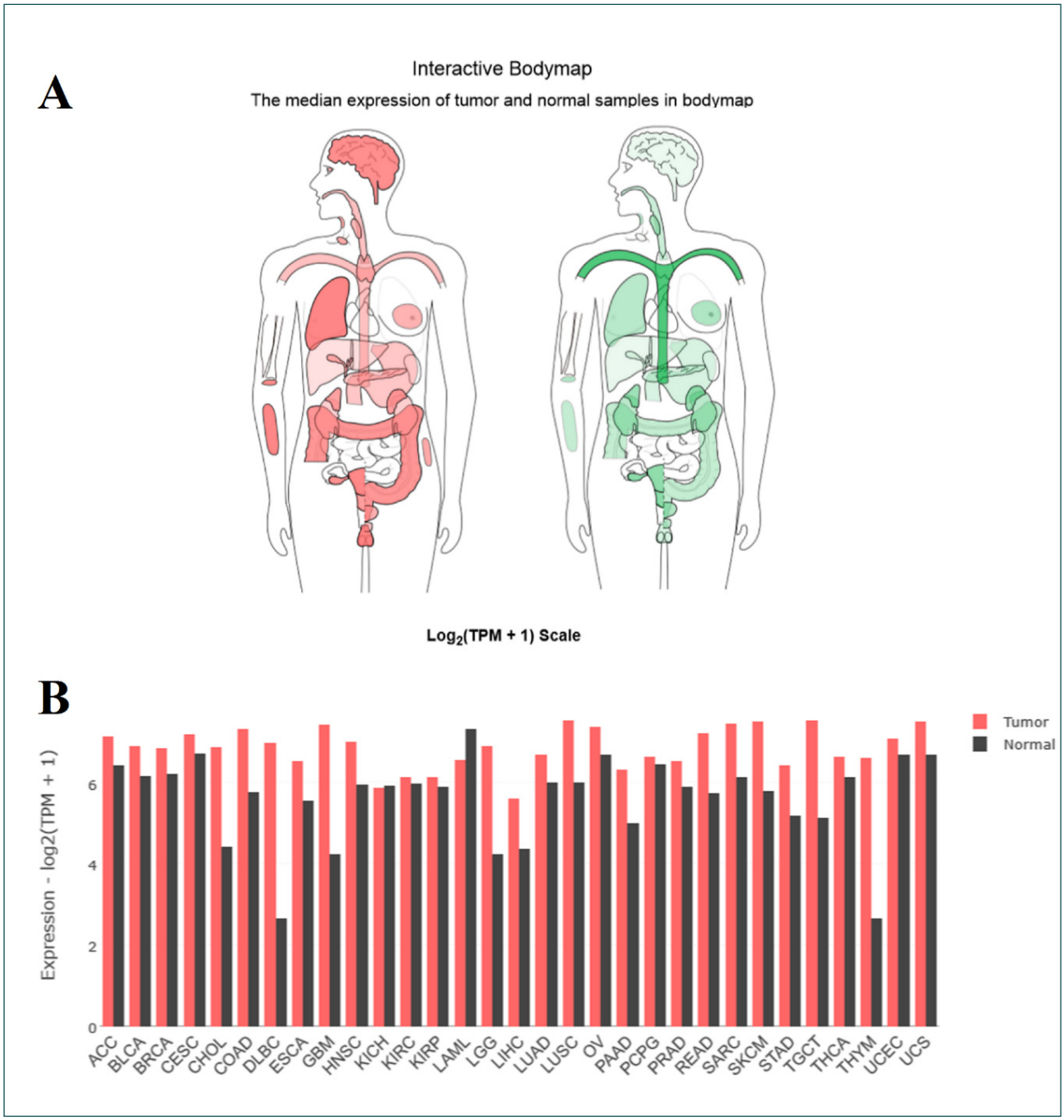

Figure 4: Expression of CDK4 across TCGA cancers (with tumor and normal samples) GPIA 2 database; (A) Body map of CDK 4 gene expression, expression level in normal tissue (green), expression level in cancerous tissue (red). (B) CDK 4 gene expression bar plot, expression level in normal tissue (black) and expression level in tumor tissue (red).

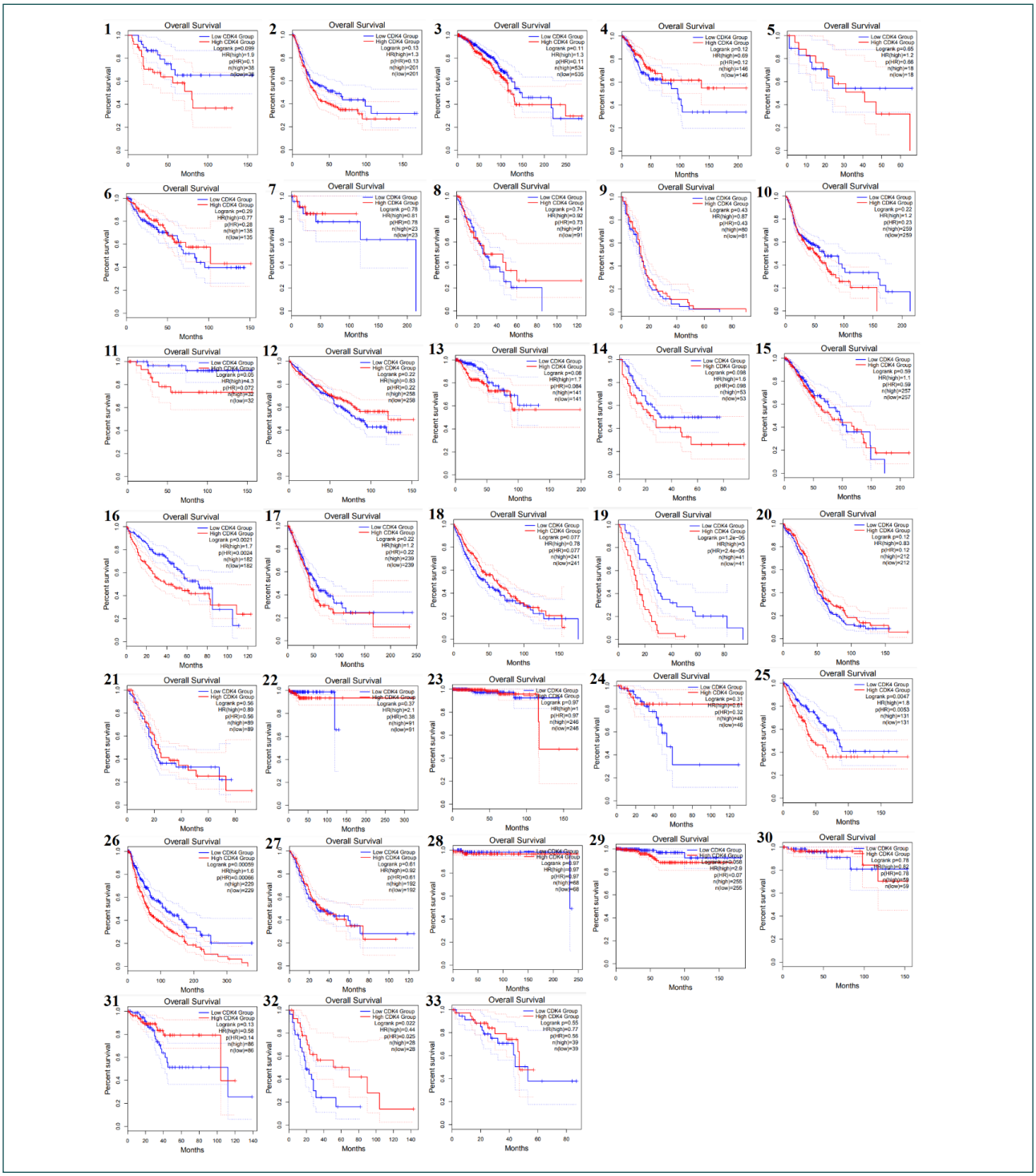

Figure 5: The relationship between the expression of this CDK4 and the survival of patients among different types of cancer; The red bar indicates the high expression of CDK 4 and the blue bar indicates the low expression of this protein in patients; (1) ACC; (2) BLCA; (3) BRCA; (4) CESC; (5) CHOL; (6) COAD; (7) DLBC; (8) ESCA; (9)GBM; (10) HNSC; (11) KICH; (12) KIRC; (13) KIRP; (14) LAML; (15) LGG; (16) LIHC; (17) LUAD; (18) LUSC; (19) MESO; (20) OV; (21) PAAD; (22) PCPG; (23) PRAD; (24) READ; (25) SARC; (26) SKCM; (27) STAD; (28) TGCT; (29) THCA; (30) THYM; (31) UCEC; (32) UCS; (33) UVM.

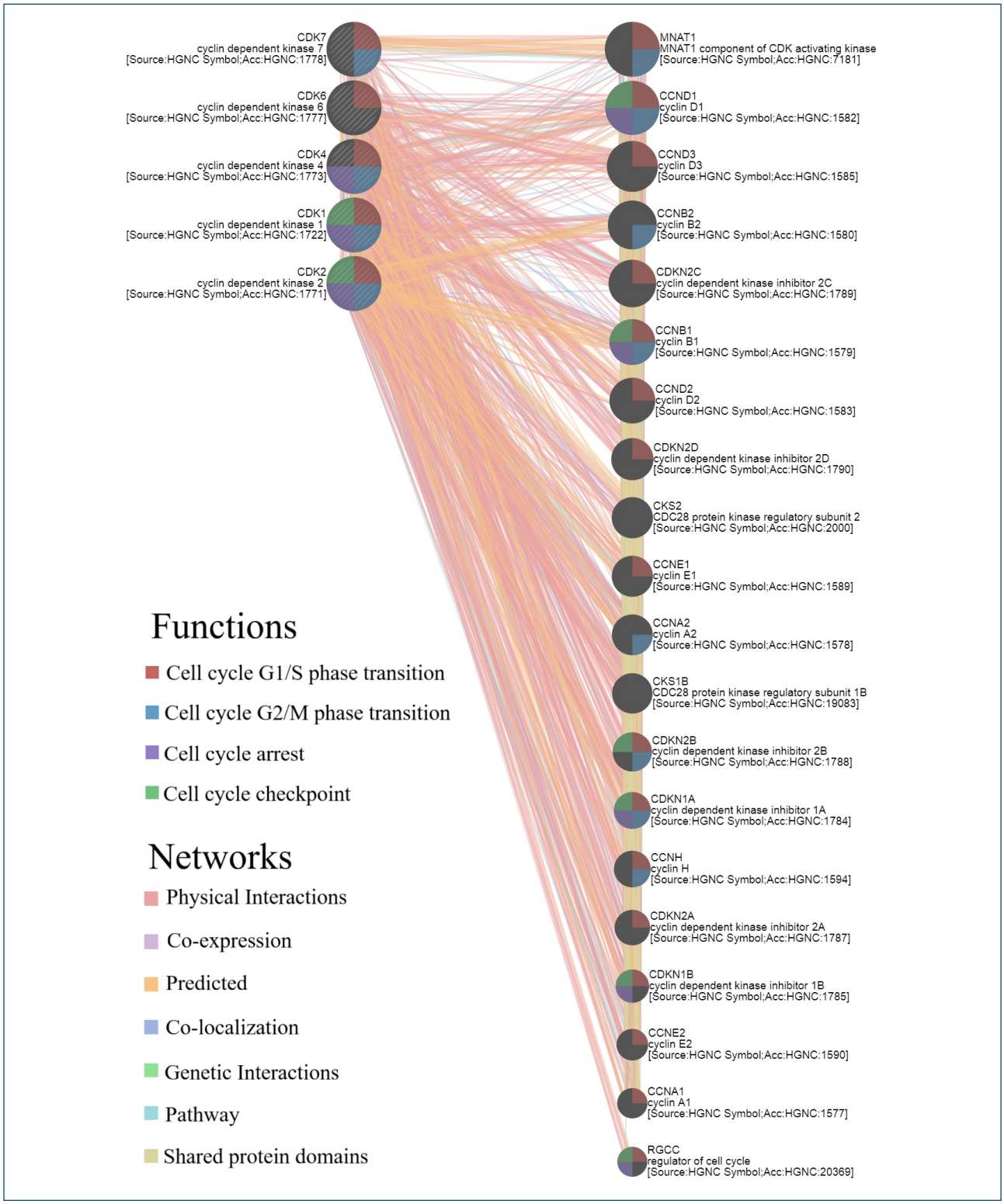

Figure 6: Network of protein interactions of CDKs 1, 2, 4, 6, and 7 and other related proteins using GeneMANIA online tool.

Table 1. Characteristics of compounds used in molecular docking.

PubChem ID	Molecular Formula	Molecular Weight
5318262	$C_{16}H_{12}O_6$	300.26 g/mol
102458506	$C_{18}H_{16}O_3$	280.3g/mol
9882773	$C_{16}H_{12}O_6$	300.26g/mol
44257552	$C_{17}H_{14}O_6$	314.29g/mol
15816423	$C_{15}H_{10}O_6$	286.24g/mol
85706708	$C_{15}H_{10}O_6$	286.24g/mol
129685938	$C_{15}H_{10}O_5$	270.24g/mol
129826856	$C_{16}H_{12}O_4$	268.26g/mol
195328	$C_{17}H_{12}O_6$	312.27g/mol
3010628	$C_{18}H_{12}O_6$	324.3g/mol
11325732	$C_{19}H_{16}O_7$	356.3g/mol
164686341	$C_{15}H_8Cl_2O_2$	291.1g/mol
56964615	$C_{16}H_{11}ClO_2$	270.71g/mol
46179803	$C_{15}H_8ClFO_2$	274.67g/mol
102578537	$C_{17}H_{15}NO_2S$	297.4g/mol
11301443	$C_{17}H_{15}NO_5$	313.3g/mol
136690817	$C_{16}H_{13}NO_3$	267.28g/mol
71816717	$C_{15}H_{10}FNO_2$	255.24g/mol
102576094	$C_{14}H_{15}N_5O_2$	285.3g/mol
18794001	$C_{16}H_{15}N_3O$	265.31g/mol
101565634	$C_{16}H_{12}ClNO_2$	285.72g/mol
134912768	$C_{18}H_{16}FNO_3$	313.3g/mol
166090129	$C_{18}H_{21}N_5O_2$	339.4g/mol
58438221	$C_{16}H_{14}ClNO_3$	303.74g/mol
134912767	$C_{18}H_{16}ClNO_3$	329.8g/mol
9361755	$C_{15}H_{12}N_2OS$	268.3g/mol

Results:

Pan-cancer investigation of CDKs

We used the GPIA 2 database to study the pan-cancer of the CDK family (CDK 1 to 7) and also to examine the expression level of these proteins in tumor and normal tissues of different types of cancers. And we drew the heat map (Figure 2). In addition to the GPIA 2 database, we also used two other databases, ULCAN and Timer 2.0, to further investigate the expression of CDK 4 in normal and tumor tissue (Figure 3 and 4). Furthermore to examining the level of expression of CDK 4, we investigated the relationship between the expression of this CDK and the survival of patients among different types of cancer (Figure 5).

Investigation of protein-protein interaction

To investigate the protein interactions of CDKs 1, 2, 4, 6, and 7, we used the online tool GeneMANIA, which shows the relationship between input CDK genes and their predicted links to other function-related genes (Figure 6).

Molecular Docking

Table 2 shows the binding affinities of the compounds studied to the ATP-binding site of CDK4 calculated by the CB-Dock2 server with the AutoDock Vina engine. The binding energies of the top-ranked neoflavonoid compounds were 3010628 (-10.8), 102458506 (-10.5), and 195328 (-10.4).

To visualize the spatial orientation of these ligands in the active site, a three-dimensional model of the binding poses was produced. Figure 8 represents the ATP-binding pocket of the CDK4/Cyclin D3 complex with the poses of the reference inhibitor Abemaciclib and the top three neoflavonoid compounds.

The two-dimensional interaction diagrams of the detailed intermolecular interactions are given in the following figures. The known CDK4 inhibitors are illustrated with their interactions in Figure 7 and the interactions of the neoflavonoid compounds are illustrated in Figures 10 and 11.

Discussion:

Cancer is one of the toughest problems in the contemporary healthcare system, as it is one of the primary causes of death on the global level, and it causes significant economic and social costs to societies [1]. Although there have been impressive improvements in treatment modalities such as surgery, chemotherapy, radiotherapy and targeted therapies, drug resistance and recurrence of the disease still remain a significant challenge to effective management of cancer [2, 3]. This has led to the emergence of innovative therapeutic approaches that have unique mechanisms of action and better safety profiles emerging as a top priority in oncology research.

The key to these problems is the very essence of cancer uncontrolled cell growth. This pathophysiological process is based on the malfunction of the specific regulatory processes which control the cell cycle. The sequential nature of the cell cycle - G1, S, G2, and M - is regulated by a complex of essential proteins, such as cyclin-dependent kinases (CDKs) and their regulators, cyclins [4-6] as shown in Figure 1. The CDK4/6-cyclin D complex is one of these regulators, and it is central to the regulation of the G1/S checkpoint, as it is a so-called gatekeeper that decides whether the cell will proliferate or go into quiescence.

We performed a systematic pan-cancer analysis of CDK1 using the levels of CDK1, CDK2, to CDK7 expression to determine which of the members of the CDK family has the highest potential to serve as a therapeutic target. In our overall analysis with the GEPIA2 database, which is presented in a heatmap in Figure 2, we found an interesting pattern of expression: out of all the CDKs analyzed, CDK4 had the highest levels of expression in the vast majority of cancer types relative to their respective normal tissues (T vs. N). This widespread and pronounced overexpression, which is obvious in the heatmap, made CDK4 a promising cancer therapeutic target.

In order to ascertain the accuracy and reliability of this finding, we re-examined the level of CDK4 expression with two other independent and strong databases: TIMER 2.0 and UALCAN. Findings of these databases as illustrated in Figures 3 and 4 gave conclusive results that validated our initial findings. TIMER 2.0 data clearly showed that CDK4 is overexpressed in cancers (GBM, DLBC, and THYM) whereas UALCAN and GEPIA2 data showed the same trend in a wider variety of malignancies. This high overlap of the results of independent data sources was a strong argument in favor of the choice of CDK4 as the main theme of our present research.

CDK4 is not only significant in its expression levels. The survival analysis conducted with GEPIA2 (Figure 5) showed that the high level of CDK4 expression correlates with the worse prognosis and reduced survival rates in

Table 2. Binding affinity of studied compounds in order of the most negative; Abemaciclib, Flavopiridol, and Rohitukine are known CDK 4 inhibitor compounds that were investigated as positive controls.

Compound	Affinity
Abemaciclib	-11.5
3010628	-10.8
102458506	-10.5
195328	-10.4
Flavopiridol	-10.3
56964615	-10.2
46179803	-10.1
129826856	-10.0
5318262	-9.9
164686341	-9.9
101565634	-9.9
129685938	-9.8
85706708	-9.6
102576094	-9.6
71816717	-9.5
134912768	-9.5
136690817	-9.3
166090129	-9.3
11325732	-9.2
102578537	-9.2
44257552	-9.1
9882773	-9.0
18794001	-9.0
Rohitukine	-8.9
11301443	-8.9
58438221	-8.8
134912767	-8.8
15816423	-8.7
9361755	-8.5

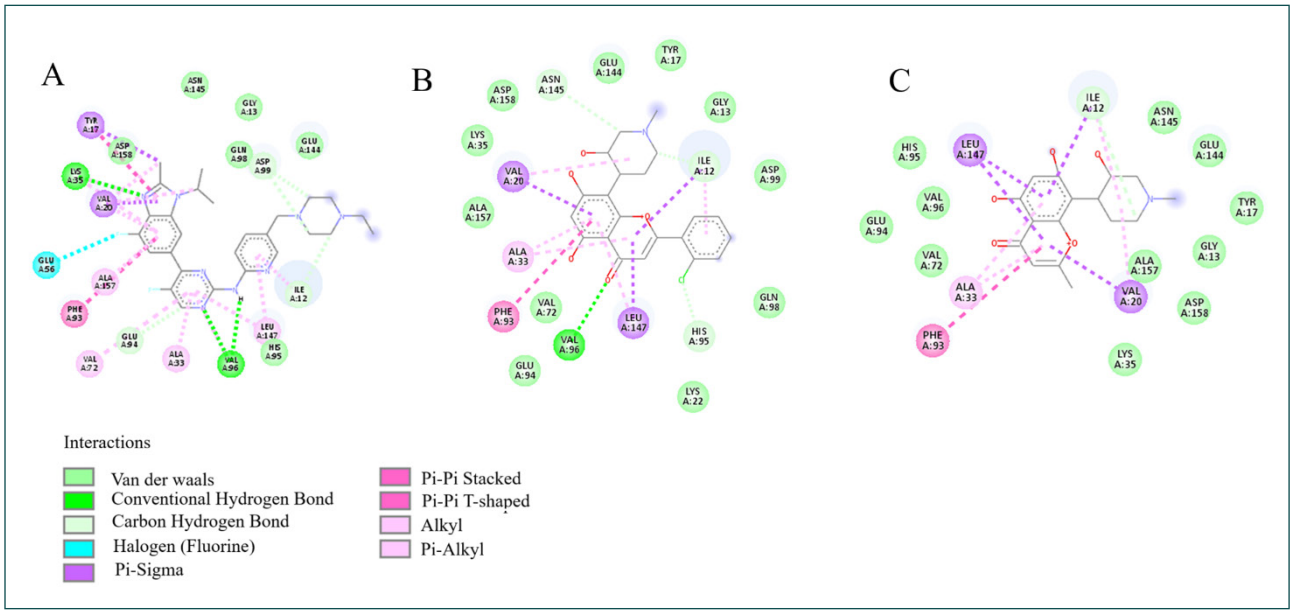

Figure 7: Intermolecular interaction of known CDK 4 inhibitors with ATP binding site in CDK 4 protein; (A) Abemaciclib; (B) Flavopiridol; (C) Rohitukine.

patients with certain types of cancer, such as ACC, BLCA,

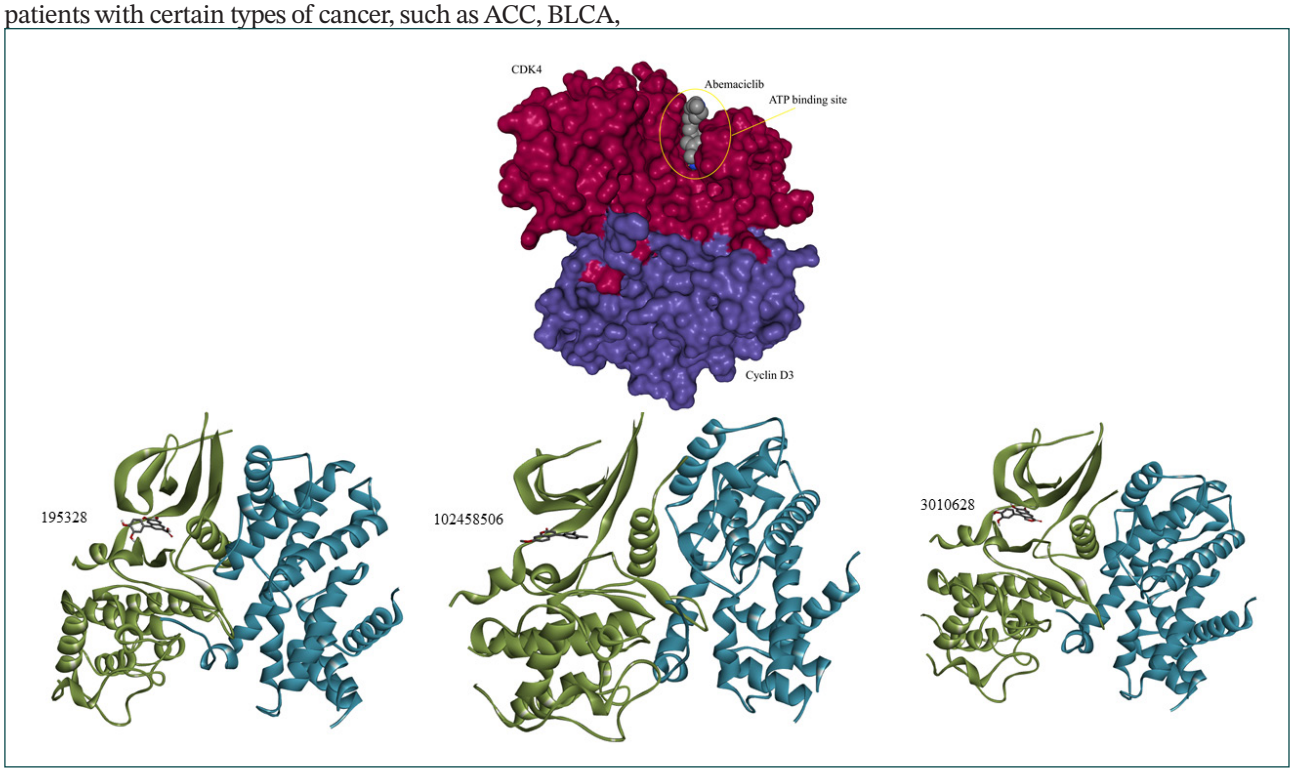

Figure 8: Three-dimensional structure of the ATP-binding site of the CDK4/Cyclin D3 complex (PDB ID: 7sj3) with bound ligands. The protein structure is depicted in the form of a molecular surface. The poses of the known CDK4 inhibitor Abemaciclib and the three highest-ranked neoflavonoid compounds, 3010628, 102458506, and 195328 are shown in the ATP-binding cavity. The visualization tools of the CB-Dock2 server and Discovery Studio Visualizer were used to generate this consolidated view, which indicates the spatial fit of all high-affinity ligands in the active site and a common binding mode that is typical of competitive ATP inhibitors.

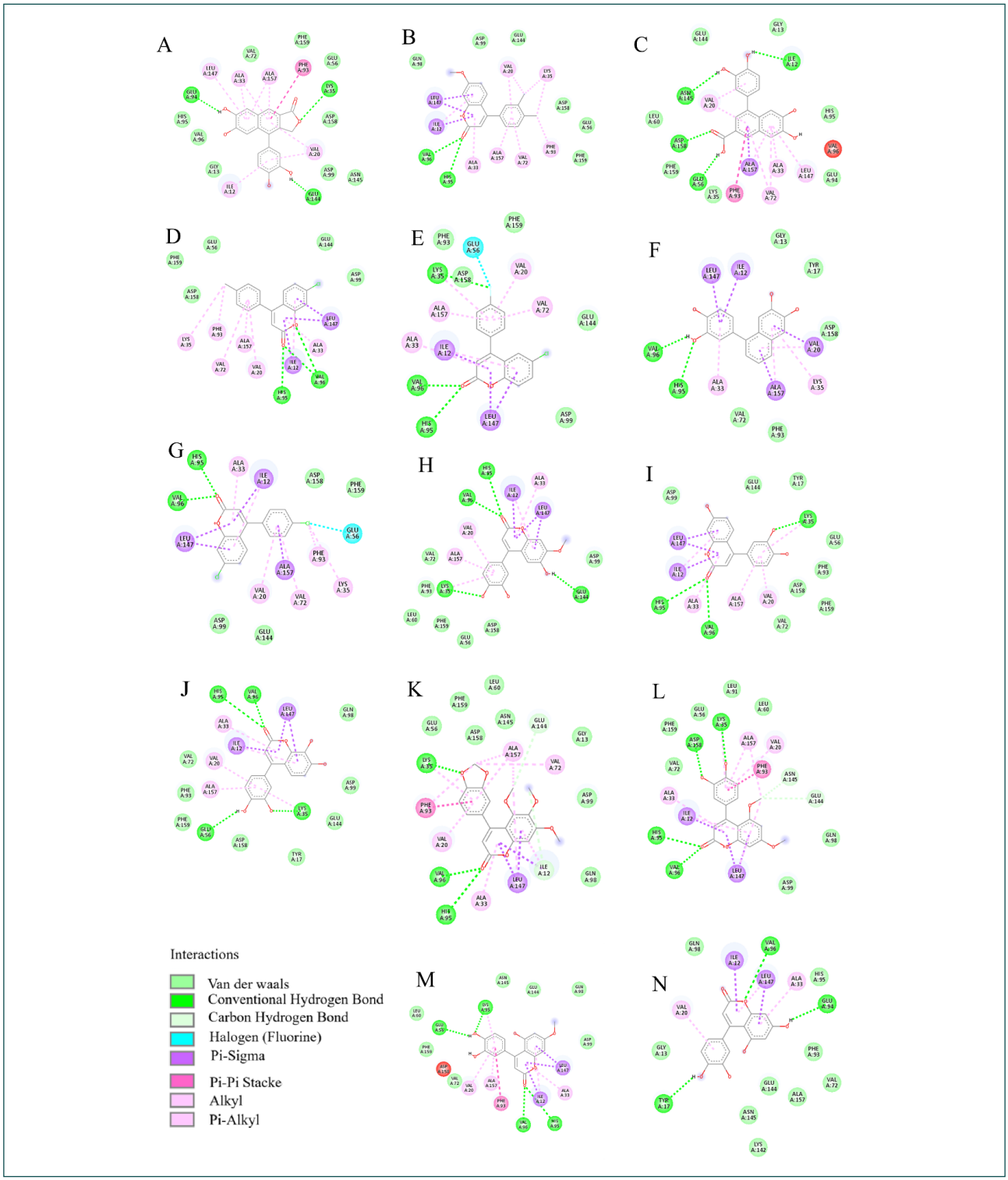

Figure 9: Interaction of neoflavonoid compounds with ATP binding site in CDK 4 protein; alphabetically from highest affinity to lowest: (A) 3010628; (B) 102458506 ; (C) 195328 ; (D) 56964615 ; (E) 46179803; (F) 129826856 ; (G) 164686341; (H) 5318262 ; (I) 129685938 ; (G) 85706708 ; (K) 11325732 ; (L) 44257552 ; (M) 9882773 ; (N) 15816423.

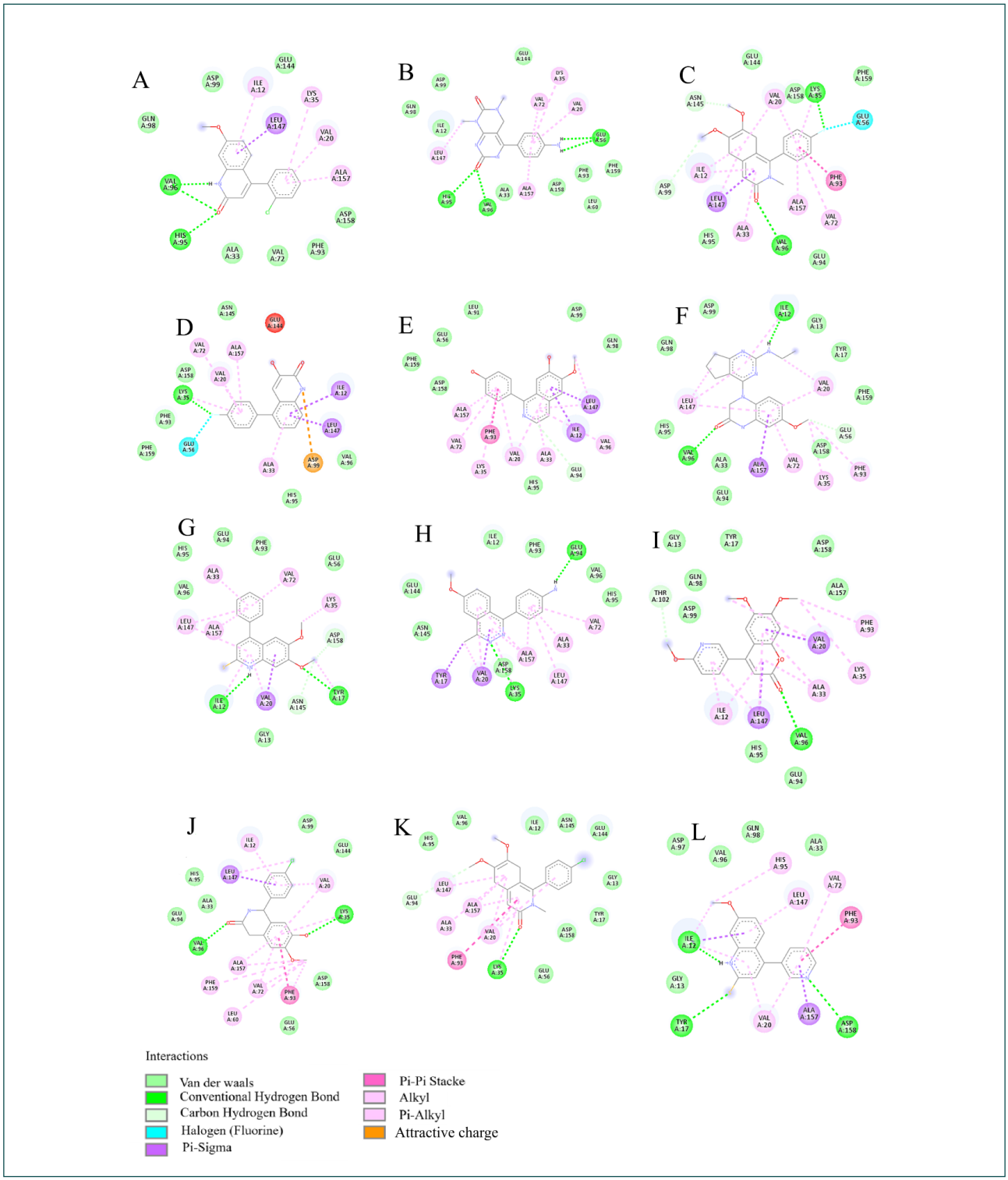

Figure 10: Interaction of similar neoflavonoid compounds with ATP binding site in CDK 4 protein; alphabetically from highest affinity to lowest: (A) 101565634; (B) 102576094; (C) 134912768; (D) 71816717 ; (E) 136690817; (F) 166090129 ; (G) 102578537; (H) 18794001 ; (I) 11301443; (G) 58438221; (K) 134912767; (L) 9361755 .

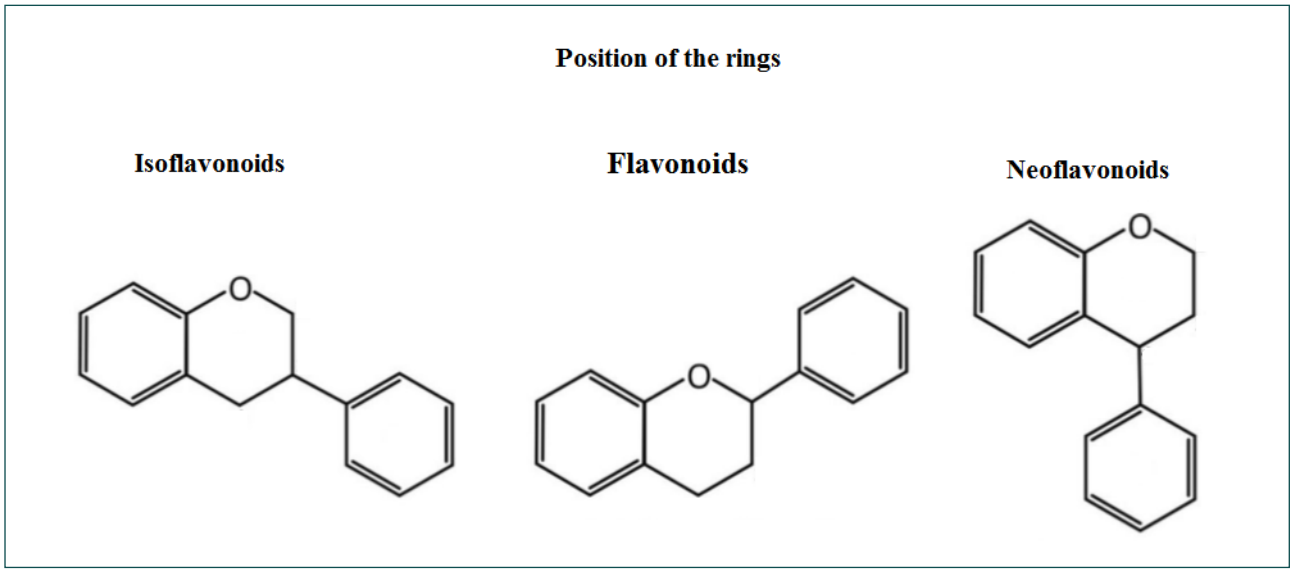

Figure 11: The position of the rings in the structure of isoflavonoids, neoflavonoids and flavonoids.

LUAD, LUSC, MESO, STAD, UCEC, and UVM. This close relationship between the expression of the CDK4 and clinical outcome supports the role of this kinase as a patient-relevant and significant therapeutic target.

In order to gain a clearer insight into the functional context of CDK4 in the cell, we mapped the protein-protein interaction network of CDK4 with the GeneMANIA tool. Figure 6 demonstrates that CDK4 is located in the middle of a complex functional network, which links it to other important CDKs (1, 2, 6, 7) and a set of genes that are associated with basic processes like cell cycle regulation, protein phosphorylation, and cyclin binding. This highly connected web of interactions highlights the critical role of CDK4 in the organization of critical cellular processes and justifies the idea that its inhibition may have a radical effect on the survival and growth of cancer cells.

The discovery of CDK4 inhibitors is a current field of cancer drug discovery. Although synthetic inhibitors such as abemaciclib have been clinically effective, natural products still offer good scaffolds in drug development. Plant-derived polyphenolic compounds, especially flavonoids, neoflavonoids, and isoflavonoids, have shown a great potential in the use as kinase inhibitors among the natural compounds. These compounds are categorized according to the positioning of their typical three-ring structure as indicated in Figure 11 [7]. The structural heterogeneity of this class is astonishing, and more than 10,000 different variants with various biological activities are known to exist [19].

These natural products that are usually ingested as plant products have demonstrated a broad range of biological activities such as antioxidant, anti-inflammatory, antimicrobial, neuroprotective, and anti-angiogenic effects [7, 8]. The most important to this study is that they have exhibited significant anti-cancer properties in different malignancies. Their therapeutic action mainly depends on the capacity to undergo certain intermolecular interactions with cellular macromolecules including receptors, enzymes and regulatory proteins. This knowledge has encouraged scientists to investigate the relationship between structure and activity and come up with synthetic analogs that have better therapeutic effects [20].

One such success story is Flavopiridol (Alvocidib), a semi-synthetic flavone analog of the natural alkaloid Rohitukine, which was isolated in Dysoxylum binectariferum. The first CDK inhibitor to be taken to clinical trials, Flavopiridol, is a breakthrough in targeted cancer therapy. It is a powerful pan-CDK inhibitor, especially of CDK1, CDK2, CDK4, CDK6 and CDK7, with competitive inhibition of the ATP-binding site. This process causes cell cycle arrest at the G1/S and G2/M phases and leads to apoptosis in different cancer cell lines [9-13].

The structural similarity between Rohitukine and Flavopiridol and the core architecture of neoflavonoids was the focus of our investigation as it is quite evident in Figure 12. This common structural motif and especially the typical chromen-4-one framework offered a very

good reason to propose that neoflavonoids could also have the same affinity with CDKs. It is on this basis that we selected a library of 28 compounds, 14 classic neoflavonoids (Figure 13) and 14 structurally analogous compounds (Figure 14), in the PubChem database.

To critically analyze their potential as CDK4 inhibitors, we conducted the molecular docking studies in order to determine their binding affinity at the ATP-binding site of CDK4. To compare them, we used three already existing CDK4 inhibitors: Flavopiridol, Rohitukine, and Abemaciclib a selective CDK4/6 inhibitor commercially available as Verzenio that has approximately 14-fold higher potency against CDK4 than against CDK6 [21, 22]. This holistic methodology allowed us to thoroughly examine the CDK4 inhibitory capability of neoflavonoids and clinically relevant reference compounds.

Our molecular docking experiments, based on the structural resemblance of known CDK inhibitors and neoflavonoids, are strong indicators of the possibility of these natural products to act as CDK4 inhibitors. The docking outcomes showed that some neoflavonoids had binding affinities that were similar to those of known

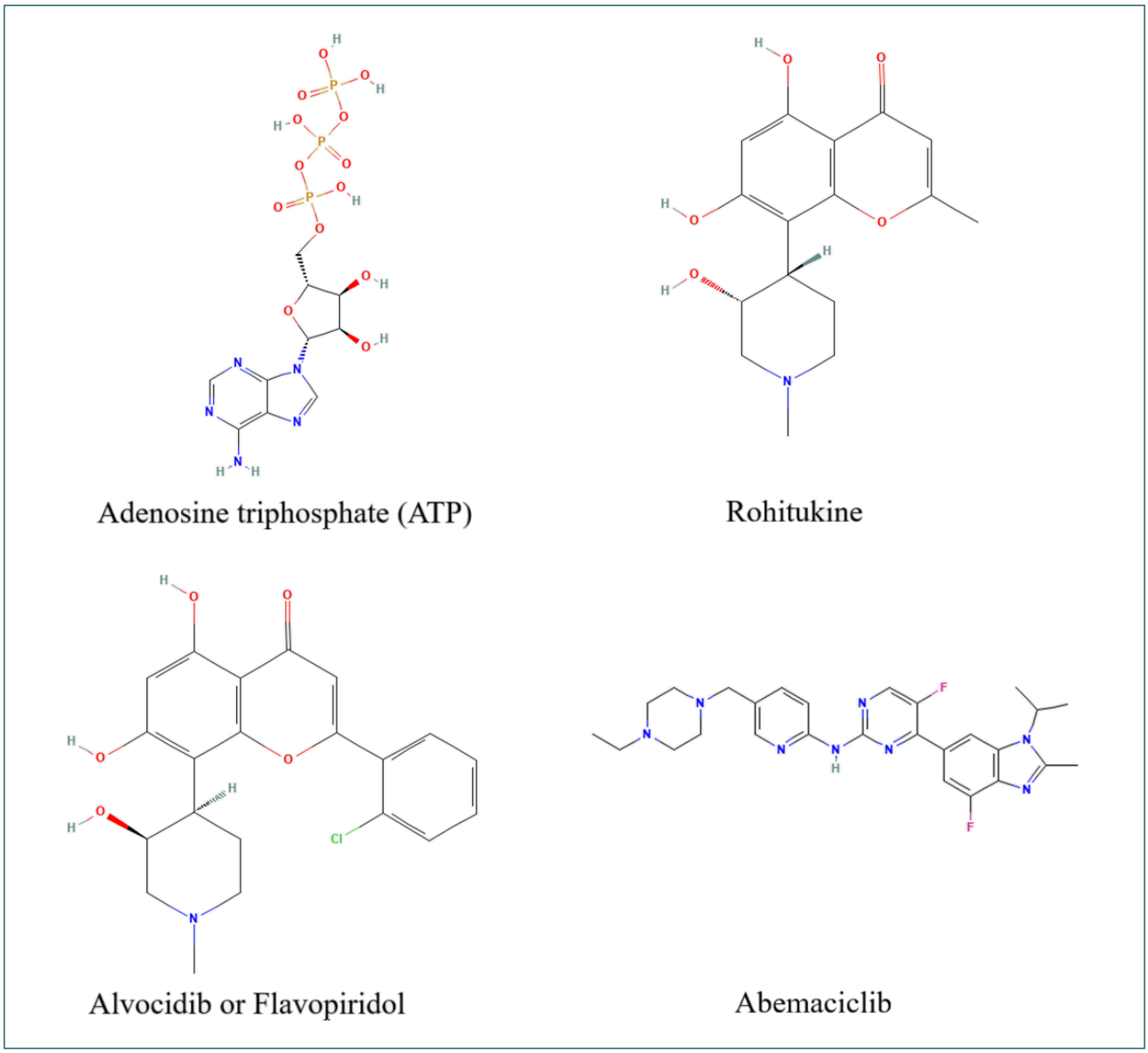

Figure 12: The structure of three known CDK4 inhibitors that inhibit this protein through binding to ATP binding site.

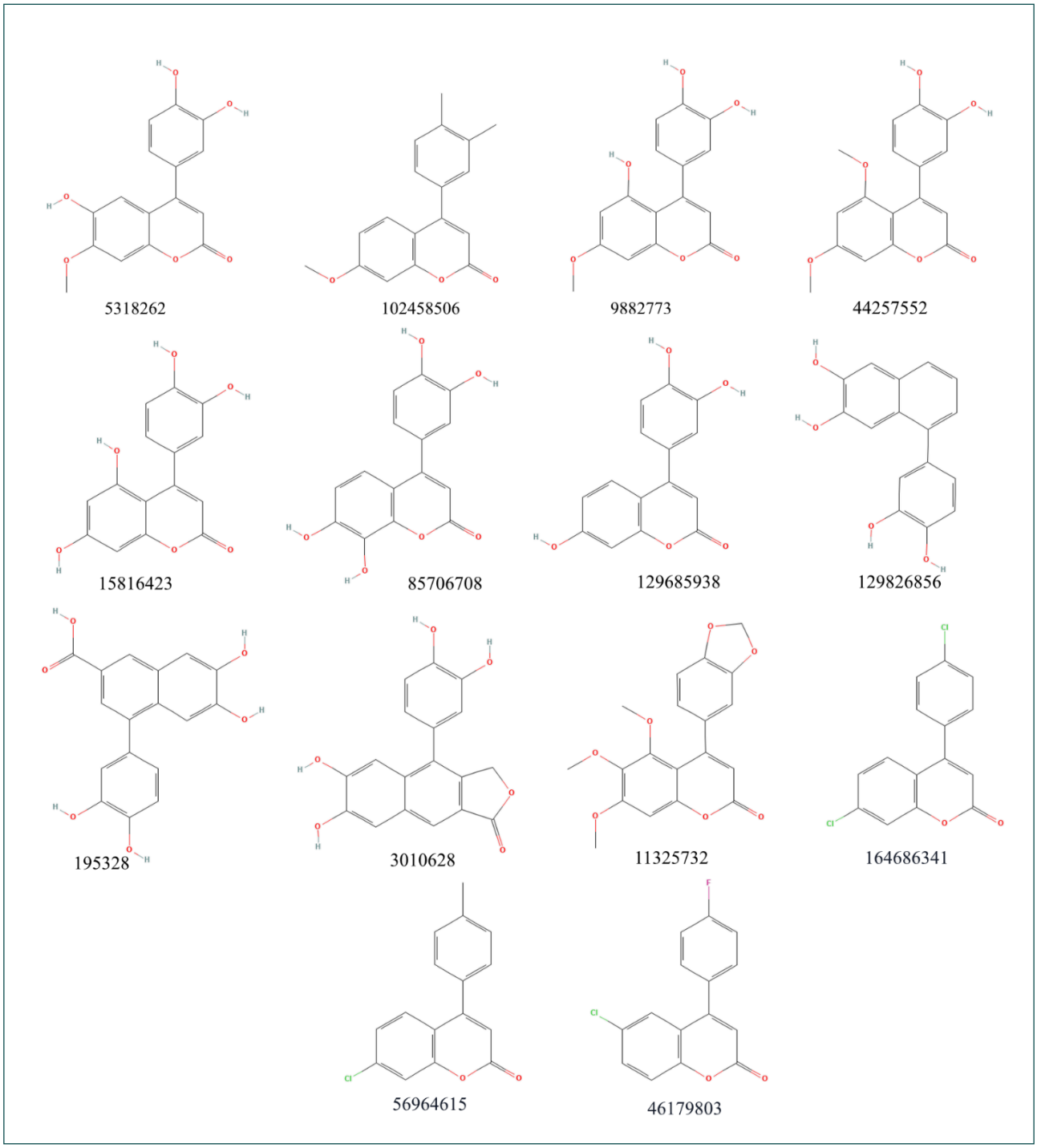

Figure 13: Chemical structure of neoflavonoid compounds; The PubChem ID of each combination is listed below.

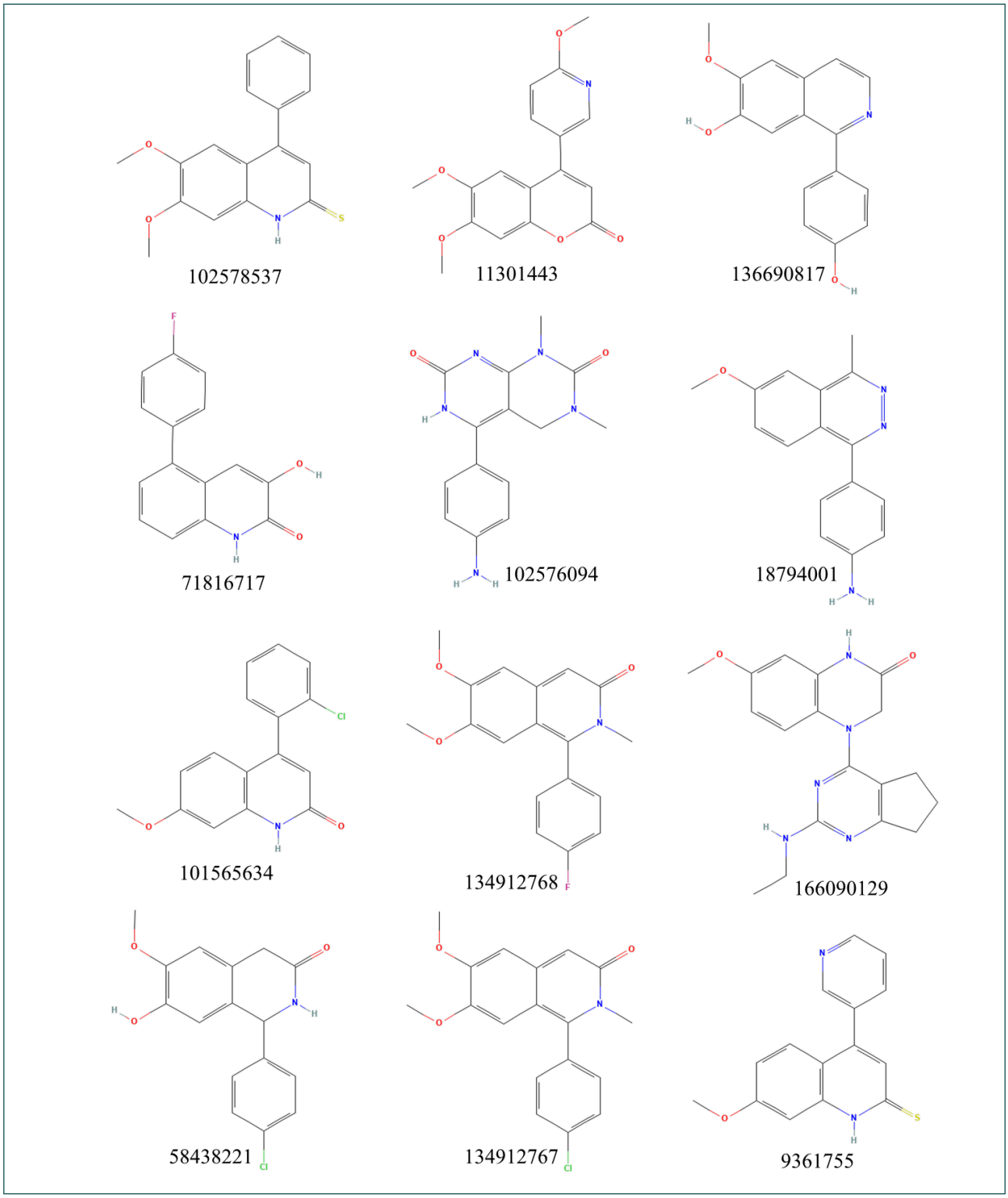

Figure 14: Chemical structure of compounds with a structure similar to neoflavonoid compounds; The PubChem ID of each combination is listed below.

inhibitors with compounds 3010628 (-10.8 kcal/mol), 102458506 (-10.5 kcal/mol), and 195328 (-10.4 kcal/mol) showing especially promising results. Nevertheless, it is essential to remember the shortcomings of the molecular docking approaches, in which energy differences of less than 1.0 kcal/mol are often within the error range of the method and cannot be regarded as statistically significant. Thus, we do not only analyze the binding energies, but the particular interaction patterns and binding modes that give us an idea about the possible mechanisms of inhibition.

The comparative study of binding modes shows that the most favorable neoflavonoids have striking similarities with Abemaciclib in their binding to important residues in the ATP-binding pocket. The overlaying structures strongly indicate that these compounds bind to the catalytic site in orientations that allow important interactions with residues that have been shown to be required to mediate CDK4 activity. Of interest is the steady interaction with Val96, Phe93, and Asp145 - residues, which are essential in binding ATP and catalysis. An example of such a compound, 3010628, shows a binding pattern that contains hydrogen bonding with Val96 and pi-pi stacking with Phe93, similar to those with Abemaciclib. In the same manner, compound 102458506 is directly hydrogen bonded with the catalytically important residue of Asp145, indicating a mechanism of action that competes directly with ATP binding.

The interaction analysis in detail gives a structural explanation of the found binding affinities and indicates that these neoflavonoids act as competitive inhibitors due to their interaction with the ATP-binding site. The preservation of the most important interactions among the highest-ranked compounds, especially between Val96 and Phe93, demonstrates that the specified residues are the points of crucial anchoring to be effective in CDK4 inhibition. Moreover, the extra contacts of these compounds with the surrounding residues including His100, Leu147, and Ala157 also make them binding specific and stable.

Although these computational findings are very encouraging, it is necessary to underline that they are not proved biological effects, but predictions. It would be necessary to experimentally verify the conclusion that these compounds cause cell cycle arrest by in vitro and in vivo experiments. Nevertheless, the structural evidence herein given gives a good basis to such investigations. The binding trends of different neoflavonoids are similar, and they interact with critical catalytic sites, which is a strong indication that the compounds could be considered as potential leads in the development of CDK4 inhibitors. The further research including molecular dynamics simulations, the calculations of binding free energy, and experimental confirmation will be necessary to completely describe the potential of these compounds and their action mechanisms.

Conclusion:

This paper has shown by extensive computational research that CDK4 is an attractive therapeutic target in a variety of cancers, with a high level of overexpression as well as association with poor patient survival. Our molecular docking findings have revealed that certain neoflavonoids especially 3010628, 102458506, and 195328 are strong putative CDK4 inhibitors with binding affinity to known inhibitors. The preserved interactions with essential catalytic residues imply a competitive inhibition mechanism of ATP-binding site. Although these computational results are a good structural evidence of the inhibition of CDK4, the therapeutic potential of these results needs to be further validated by conducting in vitro and in vivo experiments. The article has provided a base to the development of neoflavonoid-based CDK4 inhibitors as new anticancer agents.

Statement of Significance

Through a comprehensive pan-cancer analysis, high CDK4 levels were identified in various cancers, which is associated with poorer survival outcomes of patients in some cancers. This study highlights the potential of neoflavonoids as novel therapeutic agents that target CDK4, a critical cell cycle regulator associated with cancer progression. Molecular docking studies showed that several neoflavonoids bind to CDK4 with similar affinities to established inhibitors such as flavopiridol. This work highlights the therapeutic promise and molecular mechanism of neoflavonoids in cancer treatment and warrants further clinical research to elucidate the mechanisms of their efficacy.

Author statement

No conflict of interest exits in the submission of this manuscript, and manuscript is approved by all authors for publication. I would like to declare on behalf of my co-authors that the work described was review research that has not been published previously, and not under consideration for publication elsewhere, in whole or in part. All the authors listed have approved the manuscript that is enclosed.

CRediT authorship contribution statement
Mohammad-Sadegh Lotfi: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Conceptualization. Majid Jafari-Sabet: Writing – review & editing, Data curation, Writing – original draft, Visualization, Investigation, Conceptualization.

Declaration of competing interest
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Abbreviations
ACC: Adrenocortical Carcinoma
ATP: Adenosine Triphosphate
BLCA: Bladder Cancer
BRCA: Breast Cancer
CDKs: Cyclin-dependent kinases
CESC: Cervical Squamous Cell Carcinoma and Endocervical Adenocarcinoma
CHOL: Cholangiocarcinoma
COAD: Colon Adenocarcinoma
DLBC: Diffuse Large B-Cell Lymphoma
ESCA: Esophageal Carcinoma
GBM: Glioblastoma Multiforme
GTEx: Genotype-Tissue Expression
HNSC: Head and Neck Squamous Cell Carcinoma
KICH: Kidney Chromophobe
KIRC: Kidney Renal Cell Carcinoma
KIRP: Kidney Renal Papillary Cell Carcinoma
LAML: Acute Myeloid Leukemia
LGG: Lower Grade Glioma
LIHC: Liver Hepatocellular Carcinoma
LUAD: Lung Adenocarcinoma
LUSC: Lung Squamous Cell Carcinoma
MESO: Mesothelioma
OV: Ovarian Serous Cystadenocarcinoma
PAAD: Pancreatic Adenocarcinoma
PCPG: Pheochromocytoma and Paraganglioma
PRAD: Prostate Adenocarcinoma
READ: Rectum Adenocarcinoma
SARC: Sarcoma
SKCM: Skin Cutaneous Melanoma
STAD: Stomach Adenocarcinoma
TCGA: The Cancer Genome Atlas
TGCT: Testicular Germ Cell Tumors
THCA: Thyroid Carcinoma
THYM: Thymoma
UCEC: Uterine Corpus Endometrial Carcinoma
UCS: Uterine Carcinosarcoma
UVM: Uveal Melanoma.

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