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ORIGINAL ARTICLE

In silico evaluation of microRNAs in kidney renal clear cell carcinoma and drugs effects in increasing apoptosis by docking

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ABSTRACT

Background: Kidney renal clear cell carcinoma account for 2-3% of the global cancer burden and has the highest death rate of any genitourinary malignancy. Apoptosis is one of the natural immune cycles that fight against cancers. MicroRNAs are small non-coding RNA molecules that can influence the function of proteins.

Methods: The functional cycles of four microRNAs (133b, 155, 185, 217) involved in kidney cancer apoptosis were identified. Then, the effects of 7 selected medications which is routinely used by specialist doctors to treat cancer (Cabozantinib, Doxorubicin, Everolimus, Nelarabine, Sorafenib, Sunitinib, and Temsirolimus) on the target proteins were evaluated with dynamic techniques and molecular docking. Docking with powerful servers was evaluated in terms of the possibility of occurrence. Finally, the chemical and medicinal properties (Admet), toxicity, mutagenicity, and site of action of the selected drugs were predicted in silico.

Results: The results indicated that drug Sunitinib had the best binding energy with 5 target proteins (VHL= -5, VEGF-A= -7.1, BACH1= -7.4, CUL4B= -5.1, JAK-2= -8 (kcal/mol)) and showed acceptable results in terms of molecular weight (398.5 Dalton), site of action (Mitochondria), and mutagenicity (0.5200 (negative)).

Conclusion: The identified evidence demonstrates the positive efficacy and effectiveness of sunitinib in various kidney carcinomas. Targeted therapies are steps towards the targeted increase of apoptosis and the control of cell proliferation and migration, which enable the correct treatment of renal clear cell carcinoma and the prescribing of drugs that cause the least secondary damage to patients.

KEYWORDS

Kidney renal clear cell carcinoma, In silico, Apoptosis, microRNA, Molecular docking.

1. Introduction

Cancer occurs as a result of the uncontrolled division of cells due to genetic and environmental disorders. Four groups of genes play a major role in directing and creating cancer cells, which include programmed death genes, tumor suppressor genes, DNA repair genes, and oncogenes. In case of harmful genetic mutation, normal cells go out of their normal growth path and go towards the creation of tumor cells (Hosseini *et al*., 2021; Sonnenschein & Soto, 2008). Renal cell carcinoma (RCC) is a malignant tumor of the renal tubular epithelial cell system that develops in kidney tissue. One of the most prevalent cancers of the urinary system, it is also known as kidney cancer. According to US cancer statistics from 2022, kidney cancer accounts for 164,190 new cases per year and 31,990 fatalities (Bray *et al*., 2018; Tang *et al*., 2022). There are three main subtypes of RCC: kidney renal clear cell carcinoma (KIRC), kidney chromophobe (KICH), and kidney renal papillary cell carcinoma (KIRP). KIRC accounts for 75–80% of all RCC (Bray *et al*., 2018). More than 30% of kidney cancer patients develop metastases after diagnosis since the early clinical indications of the disease are relatively hard to identify. Furthermore, the treatment of individuals with kidney cancer with radiotherapy, chemotherapy, and endocrine therapy is not optimum. Although surgery is the only treatment option, 20% of patients still have metastasis and recurrence following surgery (Bex *et al*., 2010; Ljungberg *et al*., 2011). Therefore, developing an accurate and reliable risk model has become an important research direction to improve the prognosis of renal cancer. Higher standards are placed on clinical diagnosis and treatment due to advancements in precision medicine. Because clinicians may utilize prognostic models to quickly act on high-risk patients while avoiding overtreating low-risk patients, the development of prognostic models has become more and more important in clinical cancer care (Cardoso *et al*., 2019).

The advancement of technology in the field of medicine has resulted in a shift in biomedical data. As an example, the methods of determining genetic patterns have caused the creation and production of a lot of data for each person. These data have completely changed our understanding of biological processes and diseases such as cancer; These developments have made valuable data in which the number of features is greater than the number of observations, which is known as high-dimensional data. the data obtained from microarray technology in which genes (gene expression) are characteristics and patients are its observations; Data set with high dimensional variables. When the survival results are available for these patients, then the genes related to the survival of the patients can be identified. Features identified as associated with survival are then examined in experiments to better understand the biological processes that lead to the outcome of the disease. Also, these features can be used to predict survival or classify the survival of new patients (Witten & Tibshirani, 2008). Providing targets for new drug developments can also be another benefit of finding survival-related genes (Hastie *et al*., 2009).

Apoptosis is a set of intracellular events that result in cell death. Apoptosis changes the morphological shape of the cell, shrinks the cell volume, shrinks the nucleus, fragments chromatin, and destroys adhesion, which is destroyed by macrophage attack. Bcl-2 family proteins and Bcl-xl as pro-apoptotic protein and anti-apoptotic protein regulate apoptosis at the cellular level (Bax, Bad, Bid) (Czabotar *et al*., 2014). Caspases are a

class of cysteine proteases that are key regulators of apoptosis and can be found in most cells in an inactive state. Induction of apoptosis by cell death receptors results in the activation of initiator caspases (caspases 8 and 13), which is followed by the activation of downstream caspases and the formation of a proteolytic cascade. For the transmission of the intracellular signal of apoptosis, two pathways have been identified: 1- External pathway and 2- Internal path (Man & Kanneganti, 2016).

A rare kind of dominantly inherited cancer syndrome called Von Hippel Lindau (VHL) illness is predisposed to in humans by mutations in the tumor suppressor protein VHL. Throughout their lives, the patients have had recurring cysts and tumors in several tissues and organs that have the potential to turn malignant (Gossage *et al*., 2015). Research suggests that autocrine VEGF signaling in tumor cells has a significant role in increasing their proliferation and preventing apoptosis, in addition to the well-known effects of VEGF on angiogenesis. The co-expression of VEGF and its receptors has been shown in a wide range of human tumor types (Hamerlik *et al*., 2012). The transcription factor BTB and CNC homology 1 (Bach1), controls the expression of genes related to apoptosis, the oxidative stress response, mitotic chromatin dynamics, and the cell cycle. showed in mouse models of lung, liver, intestinal, pancreatic, and cardiovascular illness, Bach1 deficiency may guard against oxidative tissue damage. Endothelial cells (ECs) are less likely to proliferate, migrate, and form tubes when Wnt/-catenin signaling is disrupted by Bach1 (Wang *et al*., 2016). A part of the Cullin 4B-Ring E3 ligase complex (CRL4B), known as cullin 4B (CUL4B), is responsible for proteolysis. In terms of cancer, CUL4B is overexpressed several cancer types, such as hepatocellular carcinoma, cervical carcinoma, and osteosarcoma (Bosu & Kipreos, 2008). In the nucleus, STAT-3 dimerizes after being phosphorylated by Janus-activated kinases (JAKs), where it triggers the transcription of target genes. The regulation of cell proliferation, differentiation, and death have all been linked to STAT-3 activation with cancer development being one of the main areas of focus (Fu *et al*., 2012).

Cabozantinib targets kidney cancer by stifling tumor blood vessel growth and obstructing key signaling pathways, offering an intricate approach to treatment (Maroto *et al*., 2022), Doxorubicin, a potent anthracycline anticancer agent, intercalating DNA strands, hampering replication, and triggering apoptosis (Xu *et al*., 2022), Everolimus, an mTOR inhibitor, intricately thwarts cancer by disrupting malignant growth signals and stifling aberrant cellular proliferation (Babiker *et al*., 2019), Nelarabine, a novel purine analog, by disrupting cancerous cell DNA synthesis, presenting a multifaceted therapeutic approach (Gandhi *et al*., 2006), Sorafenib, a multi-targeted kinase inhibitor, disrupts cancer by intricately suppressing angiogenesis and crucial growth pathways (Keating, 2017), Sunitinib, a robust tyrosine kinase inhibitor, intricately thwarts cancer by disrupting tumor vascularization and crucial signaling pathways (Qi *et al*., 2020), and Temsirolimus, an mTOR inhibitor, intricately disrupts cancer progression by impeding aberrant cell growth and hampering signaling pathways vital for tumor development (Aghajanian *et al*., 2018).

In silico modeling is an effective method for investigating the biological events that occur at the tissue, cellular, and subcellular levels during cancer (Lafuente-Gracia *et al*., 2021). It is simple to understand how the use of in silico tools like bioinformatics, molecular modeling, and artificial intelligence (AI) has grown significantly

over the past few decades given recent developments in computer technology and the rapid increase in structural, chemical, and biological data available on an ever-increasing number of therapeutic targets (D'Agostino *et al*., 2013; Kapetanovic, 2008; Macalino *et al*., 2015; Song *et al*., 2009).

MiRNAs are small non-coding RNAs, that are also responsible for the regulation of mRNA function, and a lot of miRNAs have been verified to play a pivotal role in the progression of renal cell carcinoma (Dias *et al*., 2017; Kim *et al*., 2016). The specific interaction between miRNAs and CircularRNAs that are involved in metastatic renal cell carcinoma has not been fully discussed; therefore, insight into the effects of miRNAs and their potential CircRNA regulators on metastasis progression may help to clarify the pathogenesis of ccRCC[1] (Xue *et al*., 2019). Cancer treatment success is increasing, owing to chemotherapy's limited efficacy and negative side effects. As a result, boosting the efficiency and lowering the toxicity of anticancer medications is a critical aspect of cancer treatment, and it is regarded as an essential strategy among medicinal chemistry researchers. Therefore, the development and production of highly effective anticancer chemicals is a top priority. These substances should be able to inhibit cancer cell development and induce apoptosis in them via various methods while having no effect on normal cell survival (Bates & Eastman, 2017; Karthikeyan *et al*., 2013). In contrast to previous studies, we utilized apoptosis-related genes to conduct a substantial number of pan-cancer analyses in our investigation. We employed cluster analysis to successfully classify KIRC patients into several groups. We believe that the outcomes of these studies have provided meaningful and reliable data for future scientific and clinical research.

2. Materials and Methods

Bioinformatic data were obtained from TCGA (https://portal.gdc.cancer.gov/projects), mirdb (www.mirdb.org/), NCBI (www.pubmed.ncbi.nlm.nih.gov/), Target Scan (https://www.targetscan.org/vert_), Gene Expression (https://www.ncbi.nlm.nih.gov/geo/), MirBase (www.mirbase.org), Ualcan (www.ualcan.path.uab.edu) and Finally, the microRNAs paths shown in Figure 1 were identified.

2.1. Preparation of Protein

Using the UniProt database (https://www.uniprot.org/) and articles (Bosu & Kipreos, 2008; Fu *et al*., 2012; Gossage *et al*., 2015; Hamerlik *et al*., 2012; Wang *et al*., 2016), the proteins involved in the pathway were identified, and the necessary information was used to retrieve the protein's structure from the database, and the raw proteins structure was obtained from the PDB database (https://www.rcsb.org/) was extracted and by Discovery Studio and Chimera software, the initial preparation on this proteins means, more precisely, the removal of additional molecules such as previous ligands and water outside the structure, adding hydrogen to the structure, adding Removed residues, adding charge to amino acids and minimizing the energy of the protein's structure is done for molecular docking.

On the other hand, the important binding sites that target drugs can bind to these sites are provided by the COACH database (https://zhanggroup.org/COACH/); (accessed on 25 November 2022) and the Literature section of the PDB database (https://www.rcsb.org/) were identified and extracted to be used and evaluated in the

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final analysis. The 3D structures developed via the Phyre2 server were validated by Procheck (Available online: https://www.ebi.ac.uk; (accessed on 25 November 2022). The Procheck server was used for Ramachandran plot analysis (Gaur *et al*., 2015).

2. 2. Preparation of the Ligands

Bioinformatics Analytics and Identification of Apoptosis Pathways: miRNA-133, miRNA-155, miRNA-185, and miRNA-217 were selected as the focus of this research, as depicted in Figure 1. To achieve this, an investigation was conducted utilizing the DrugBank database (https://go.drugbank.com/) to select medications commonly prescribed by specialists for cancer treatment. The suggested drug ligands were identified through Molecular Docking. Subsequently, a comprehensive drug list was compiled using the PubChem database (https://pubchem.ncbi.nlm.nih.gov), including the IUPAC Names and Canonical SMILES notations of the drugs. The 2D structure of each drug was reimagined using ChemBio Draw software, followed by its conversion into a 3D structure with the assistance of ChemBio 3D software. The obtained 3D structures underwent Molecular Dynamics simulations and Minimized Energy calculations using the same software, enhancing their readiness for Molecular Docking. The outcomes of this process are presented in Table 1, which displays the prepared database results after software-driven preparation.

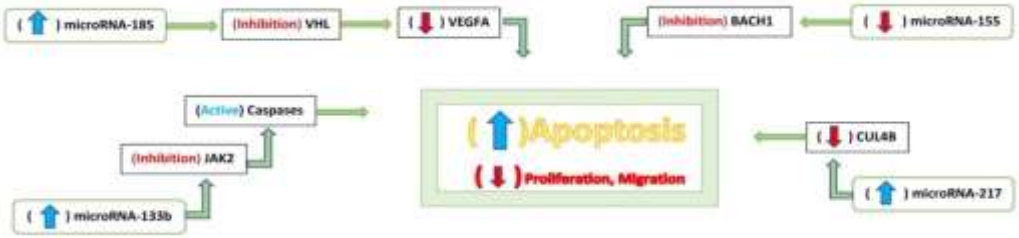
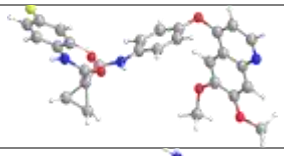
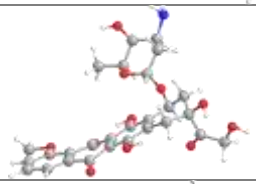
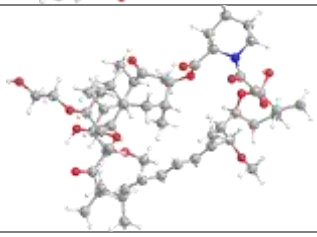
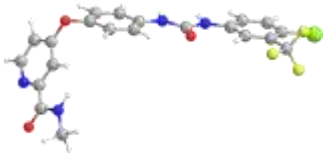
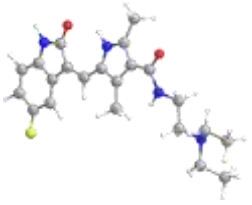
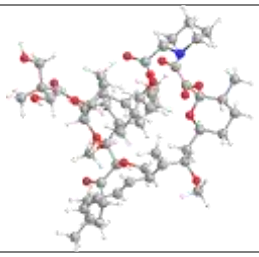


Figure 1. microRNAs pathways in the apoptosis of kidney clear cells

Table 1. Generic names, IUPAC name, PubChem (CID) & 3D structures seven selected Drug

Generic Name IUPAC Name	PubChem (CID)	3D Structure
Cabozantinib 1-N-[4-(6,7-dimethoxyquinolin-4-yl)oxyphenyl]-1-N'-(4-fluorophenyl)cyclopropane-1,1-dicarboxamide	25102847	
Doxorubicin (7S,9S)-7-[(2R,4S,5S,6S)-4-amino-5-hydroxy-6-methyloxan-2-yl]oxy-6,9,11-trihydroxy-9-(2-hydroxyacetyl)-4-methoxy-8,10-dihydro-7H-tetracene-5,12-dione	31703	
Everolimus (1R,9S,12S,15R,16E,18R,19R,21R,23S,24E,26E,28E,30S,32S,35R)-1,18-dihydroxy-12-[(2R)-1-[(1S,3R,4R)-4-(2-hydroxyethoxy)-3-methoxycyclohexyl]propan-2-yl]-19,30-dimethoxy-15,17,21,23,29,35-hexamethyl-11,36-dioxa-4-azatricyclo[30.3.1.04,9]hexatriaconta-16,24,26,28-tetraene-2,3,10,14,20-pentone	6442177	

Continued table 1. Generic names, IUPAC name, PubChem (CID) & 3D structures seven selected Drug

Generic Name IUPAC Name	PubChem (CID)	3D Structure
Nelarabine (2R,3S,4S,5R)-2-(2-amino-6-methoxypurin-9-yl)-5-(hydroxymethyl)oxolane-3,4-diol	3011155	
Sorafenib 4-[4-[[4-chloro-3-(trifluoromethyl)phenyl] carbamoylamino]phenoxy]-N-methylpyridine-2-carboxamide	216239	
Sunitinib N-[2-(diethylamino)ethyl]-5-[(Z)-(5-fluoro-2-oxo-1H-indol-3-ylidene)methyl]-2,4-dimethyl-1H-pyrrole-3-carboxamide	5329102	
Temsirolimus [(1R,2R,4S)-4-[(2R)-2-[(1R,9S,12S,15R,16E,18R,19R,21R,23S, 24E,26E,28E,30S,32S,35R)-1,18-dihydroxy-19,30-dimethoxy-15,17,21,23,29,35-hexamethyl-2,3,10,14,20-pentaoxo-11,36-dioxa-4-azatricyclo[30.3.1.04,9]hexatriaconta-16,24,26,28-tetraen-12-yl]propyl]-2-methoxycyclohexyl] 3-hydroxy-2-(hydroxymethyl)-2-methylpropanoate	6918289	

2. 3. Molecular Docking

Molecular docking is done to predict the probability of ligand-protein binding to check the result of this binding, which is the activation of the apoptosis cycle in this study. The biological macromolecule (target protein) and ligands are docked by AutoDock-Vina software, and the structures that can bind to the target protein are selected with higher quality.

2.4. Pharmacokinetic Properties Analysis

Among the different characteristics of the drug, which are caused by the diversity of the chemical properties of the drugs and the place of their effect, we have chosen the characteristics that give the best effect to the doctor in choosing the targeted treatment. Their pharmacokinetic properties were analyzed by the online databases of protein-ligand interaction profiler (https://plip-tool.biotec.tu-dresden.de/plip-web/plip/index) and admetSAR (http://lmmd.ecust.edu.cn/admetsar2/) is evaluated and an effective and targeted drug regimen is suggested.

3. Result

3. 1. Bioinformatic Prediction

Figure 2, which is the graphs obtained from the analysis of bioinformatics data extracted from the TCGA database. In parts A, B, C, and D, respectively, miRNA-133b, miRNA-155, miRNA-185, and miRNA-217 are present in KIRC based on patient and normal. The red box indicates the patient state and the blue box indicates the normal state. The expression level of miRNA-133b and miRNA-217 in normal conditions is higher than in KIRC patients (By measuring this microRNA, it shows less expression in

patients) and the expression level of miRNA-155, and miRNA-185 in normal conditions is lower than KIRC (By measuring this microRNA, it shows high expression in patients). This increase and decrease of microRNAs will be very helpful in measuring the amount of proteins according to Figure 1 in the next steps.

Four very important apoptotic pathways associated with identified microRNAs are displayed in Figure 1. These pathways can be controlled by proteins that are our medicinal target in this research. Proteins VHL, VEGF-A, BACH1, CUL4B, and JAK-2 have been selected as the target proteins in this study from the analysis of the functional cycle of microRNAs identified from bioinformatics. Uniport codes for these proteins are determined by checking the Uniport database. The PBD ID is then extracted from the PDB database and the initial preparation for molecular docking is performed on these proteins by Chimera software. On the other hand, the important binding sites where the target drugs can bind to these sites are identified by the COACH database and the Literature section of the PDB database which is shown in Table 2.

Table 2. Proteins name, UniProt code, PDB ID and Active site

Proteins name	Uniport code	PDB ID	Active site
VHL	P40337	1LM8	ASN67, TYR98, HIS110, TYR112
VEGF-A	P15692	6Z13	TRP7, PHE12, PHE17, TYR21, GLU64
BACH1	O14867	2IHC	SER17, THR18, ASN19, GLN27, ASP35, ASN117
CUL4B	Q13620	4A0C	ARG192, ASP213, LEU214, GLN236, CYS237, ALA239
JAK-2	O60674	2B7A	LEU855, VAL863, ALA880, VAL911, MET929, TYR931, GLY935, LEU983

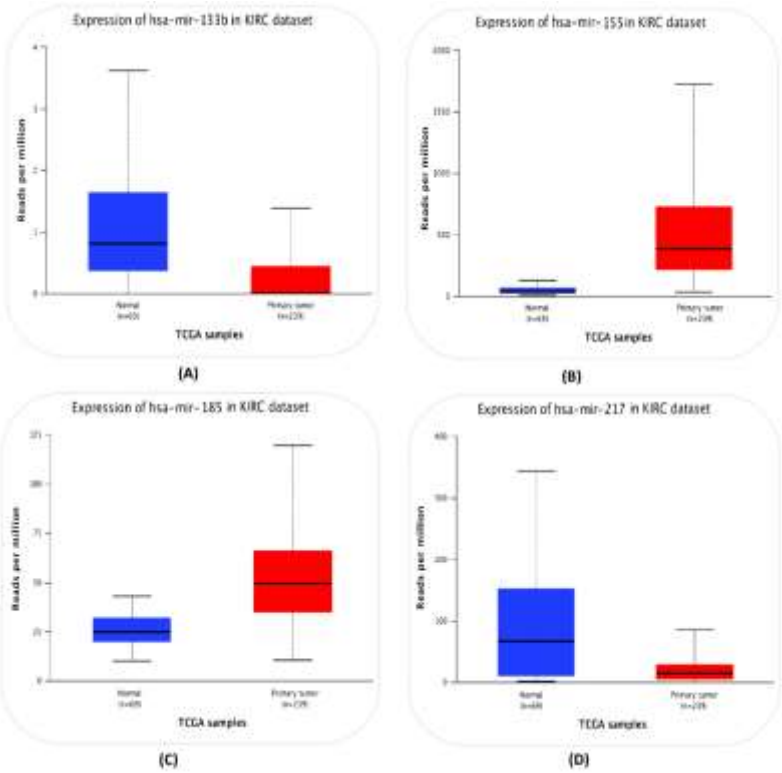


Figure 2. Expression of microRNAs in KIRC based on patient (red) and normal (blue); (A) MicroRNA-133b; (B) MicroRNA-155; (C) MicroRNA-185; (D) MicroRNA-217.

In Figure 3, Ramachandran plot analysis was performed by the Procheck server (Rahman *et al*., 2014). According to the Ramachandran plot, which shows the results of proteins 1LM8, 6Z13, 2IHC, 4A0C, and 2B7A respectively (92.2%, 94.2%, 91.8%, 83.9%, 91.4%) of residues are in the most favored region, (6.9%, 4.1%, 8.2%, 14.2%, 8.0%) in the additional allowed region, (0.3%, 1.7%, 0%, 1.1%, 0.4%) in the generously allowed, and (0.7%, 0%, 0%, 0.7%, 0.2%) in the disallowed region. The Ramachandran plot quantified that the quality of the model is good if over 90 % of residues are in the most favored regions.

3. 2. Molecular Docking

After docking with AutoDockVina, the results of binding affinity Auto Dock (KCAL/MOL) are shown in Table 3 (The cut-off has been set at the binding affinity of "-5 kcal/mol", The sign (*) in the table means that an acceptable link has not been created in the desired positions).

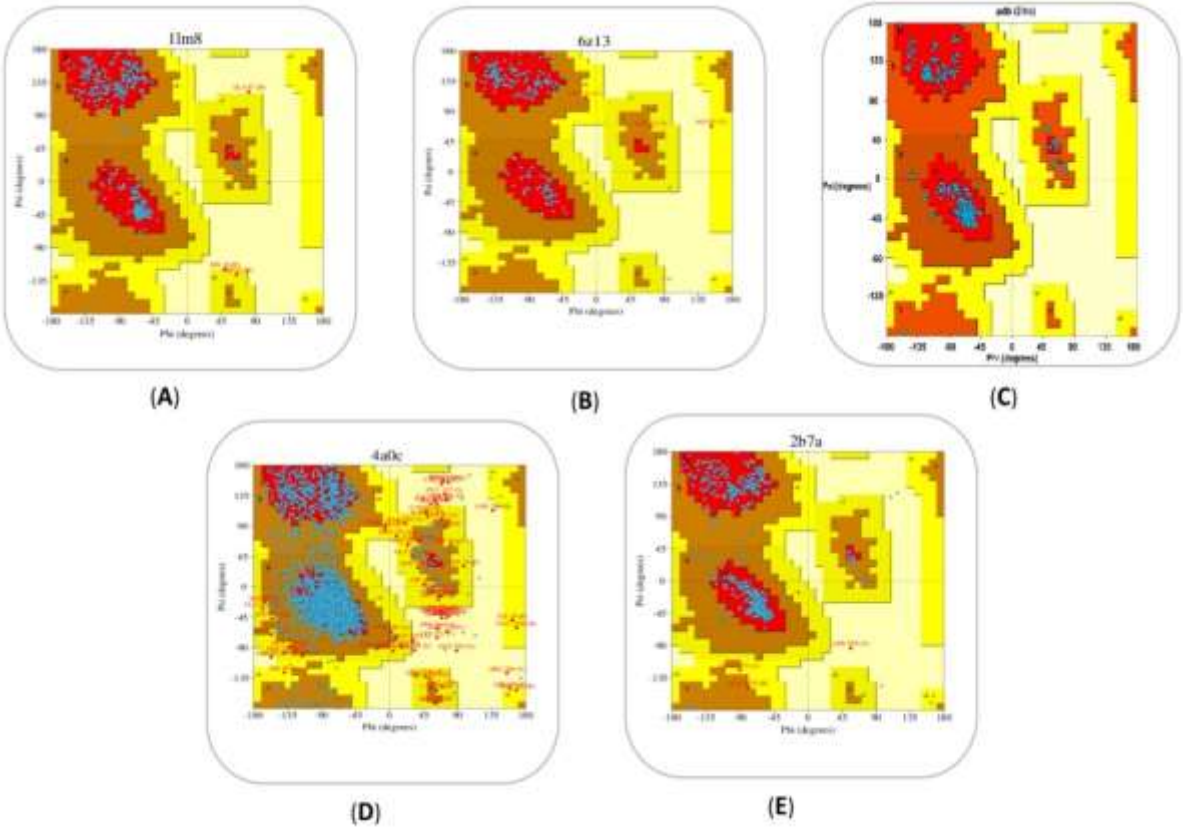


Figure 3. Ramachandran plot analysis shows the schematic structure of the protein. (A) 1LM8; (B) 6Z13; (C) 2IHC; (D) 4A0C; (E) 2B7A.

Table 3. The results of Auto Dock Vina (binding affinity)

Name of the drug	Binding Affinity (kcal/mol)				
	1LM8	**2B7A**	**2IHC**	**4A0C**	**6Z13**
Cabozantinib	-5.7	-8.8	-8	-0.9 *	-7.6
Doxorubicin	-1.4 *	-7.6	-6.4	-1.5 *	-7.5
Everolimus	3.6 *	27.8 *	-6.1	110 *	-7.3
Nelarabine	-3.7 *	-6.8	-6.4	-4.4 *	-6
Sorafenib	-5.1	-9.5	-8.1	-1.3 *	-8.3
Sunitinib	-5	-8	-7.4	-5.1	-7.1
Temsirolimus	33.5 *	40.0 *	-5	109 *	-7.5

The binding of drugs CABOZANTINIB, SORAFENIB, and SUNITINIB to protein VHL (1LM8), leads to the inactivation of VHL and it also reduces the performance of VEGFA, and drugs DOXORUBICIN, EVEROLIMUS, TEMSIROLIMUS are ineffective in protein and pathway VHL.

The binding of all drugs evaluated in this study (CABOZANTINIB, DOXORUBICIN, EVEROLIMUS, NELARABINE, SORAFENIB, SUNITINIB, and TEMSIROLIMUS) to proteins VEGF-A (6Z13) and BACH1 (2IHC) respectively leads to the inactivation of VEGFA and BACH1.

The binding of drug SUNITINIB to protein CUL4B (4A0C), leads to the inactivation of CUL4B, and drugs CABOZANTINIB, DOXORUBICIN, EVEROLIMUS, NELARABINE, SORAFENIB, TEMSIROLIMUS ineffective in protein and pathway CUL4B.

The binding of drugs CABOZANTINIB, DOXORUBICIN, NELARABINE, SORAFENIB, and SUNITINIB to protein JAK-2 (2B7A), leads to the inactivation of JAK-2, and it also increases the performance of Caspases, and drugs EVEROLIMUS, TEMSIROLIMUS ineffective in protein and pathway CUL4B.

Based on the findings derived from the molecular docking outcomes presented in Table 3, it is evident that the drug SUNITINIB has exhibited the most favorable performance. This is evidenced by its commendable binding energy, molecular weight, site of action, and absence of mutagenicity. The potential utility of SUNITINIB is thus considerable. Additionally, two proteins, namely 2IHC and 6Z13, have demonstrated the capability to bind with a total of 7 drugs. This promising scenario bodes well for disease prognosis, as it enables physicians to adopt a more flexible approach in drug prescription. The increased array of available treatment options augments the likelihood of positive treatment responses, contributing to a more comprehensive and effective therapeutic approach.

In Figure 4, the structural model of binding of 5 proteins with drug SUNITINIB that had acceptable binding affinity (KCAL/MOL) in docking simulation was selected. 3-D protein structure, involved residues, drug ligand and Bond Length are visible.

3. 3. Prediction of medicinal properties (Admet)

Useful information for drug analysis and stratification, which is the result of bioinformatics analysis of the admetSAR and protein-ligand interaction profiler (PLIP) database are shown in Table 4 and Table 5.

According to Table 4, Two drugs DOXORUBICIN and NELARABINE have Nucleus Subcellular localization and the others have Mitochondria localization, three drugs DOXORUBICIN, EVEROLIMUS, and TEMSIROLIMUS do not pass through the blood brain barrier, while others do. The results of toxicity (Hepatotoxicity, Respiratory toxicity, Reproductive toxicity), and Ames Mutagenesis are displayed in Table 4 (In this table, the positive sign (+) means that it crosses the blood-brain barrier, it is toxicity in the liver, respiratory and reproductive, it is a mutagen, and the negative sign (-) is the opposite of the above).

Since the drug Sunitinib has the best binding energy among other drugs and according to the Admet shown in Table 4, it has a negative mutagenicity, but due to its toxicity and crossing the blood-brain barrier, it should be prescribed more carefully.

In Table 5, The number of novel binding structures that were generated between the ligand and the protein as a result of the docking findings were assessed using the Protein-Ligand Interaction Profiler (PLIP)

online database (HI= HYDROPHOBIC INTERACTIONS/ HB= HYDROGEN BONDS/ OB= OTHER BONDS (SALT BRIDGES, Π-CATION INTERACTION, …), The sign (*) in the table means There are no atomic bonds).

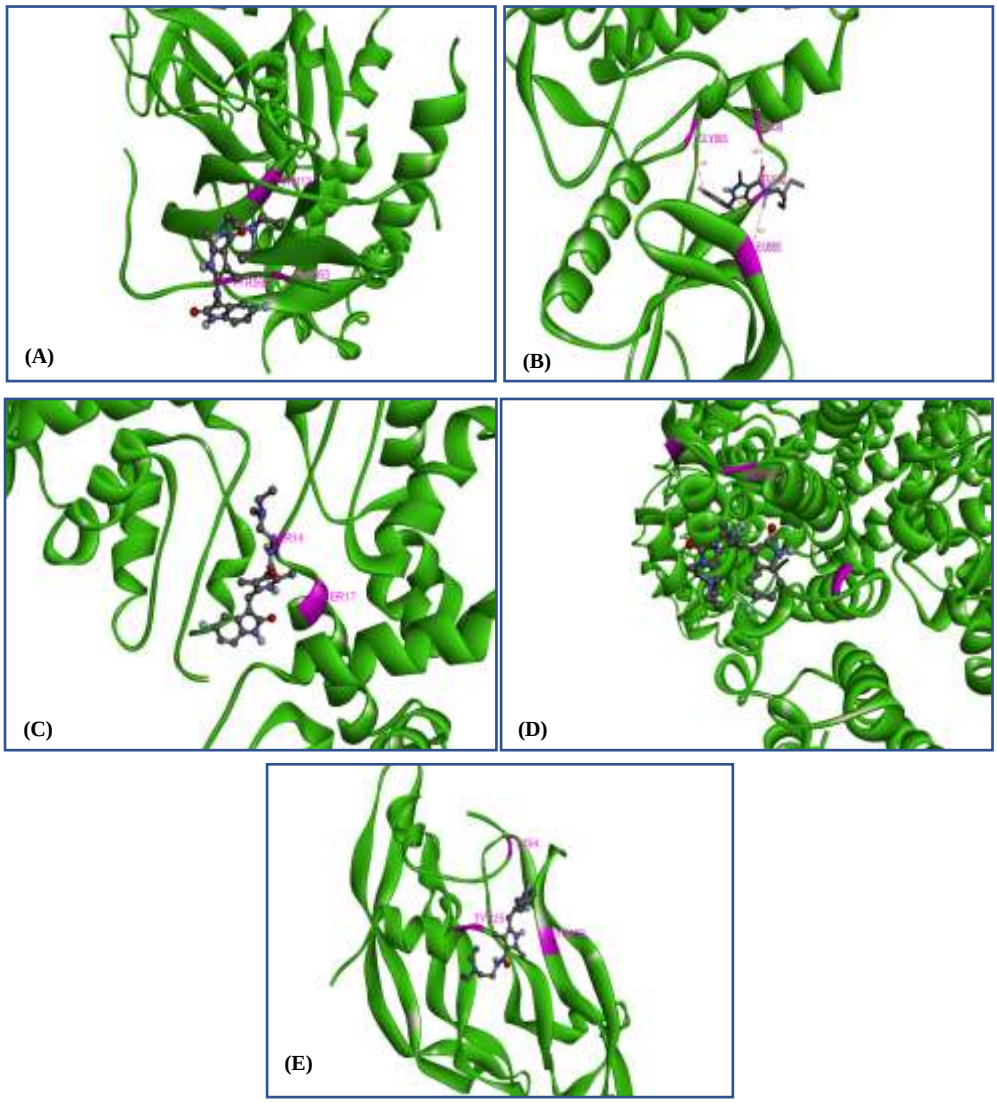


Figure 4. Structural model of docking simulation (A) 1LM8- SUNITINIB; (B) 2B7A - SUNITINIB. Structural model of docking simulation (C) 2IHC- SUNITINIB; (D) 4A0C - SUNITINIB; (E) 6Z13- SUNITINIB.

Table 4. The results of AdmetSAR database

Name of the drug	Admet SAR						
	Subcellular localization	Molecular Weight	Blood Brain Barrier	Hepatotoxicity	Respiratory toxicity	Reproductive toxicity	Ames Mutagenesis
Cabozantinib	Mitochondria	501.5	+	0.5375 (-)	0.9111 (+)	0.8444 (+)	0.5500 (-)
Doxorubicin	Nucleus	543.5	-	0.8500 (-)	0.9778 (+)	0.9778 (+)	0.9900(+)
Everolimus	Mitochondria	958.2	-	0.6250 (-)	0.9333 (+)	0.9667 (+)	0.6200 (-)
Nelarabine	Nucleus	297.27	+	0.5045 (-)	0.9889(+)	1.0000 (+)	0.6000 (-)
Sorafenib	Mitochondria	464.8	+	0.9750(+)	0.6444(+)	0.8000 (+)	0.5100 (-)
Sunitinib	Mitochondria	398.5	+	0.9750(+)	0.9333(+)	0.9333 (+)	0.5200 (-)
Temsirolimus	Mitochondria	1030.3	-	0.5032(+)	0.9222(+)	0.9333 (+)	0.5300 (-)

Table 5. The results of PLIP database

Name of the drug	Protein ligand Interaction Profiler (PLIP)														
	1LM8			2B7A			2IHC			4A0C			6Z13		
	HI[1]	HB[2]	OB[3]	HI	HB	OB	HI	HB	OB	HI	HB	OB	HI	HB	OB
Cabozantinib	2	3	*	5	4	1	*	5	2	*	*	*	5	4	*
Doxorubicin	*	*	*	2	1	3	3	6	*	*	*	*	4	7	*
Everolimus	*	*	*	*	*	*	3	3	*	*	*	*	2	3	*
Nelarabine	*	*	*	2	1	3	3	11	1	*	*	*	1	6	*
Sorafenib	1	3	*	3	3	*	1	3	*	*	*	*	4	5	*
Sunitinib	1	3	*	5	5	*	3	4	2	4	6	1	2	3	1
Temsirolimus	*	*	*	*	*	*	3	3	*	*	*	*	4	2	*

1. HI= Hydrophobic Interactions /
2. HB= HYDROGEN Bonds
3. OB= other bonds (SALT BRIDGES, π-Cation Interaction, …)

In Table 3 of the binding affinity (KCAL/MOL) and type of bonds in Table 5, which ultimately increasing apoptosis and controlling cell proliferation and migration in kidney renal clear cell carcinoma.

4. Discussion

Recently, it has been reported that miRNAs expressed in specific body compartments can be released into the blood as a consequence of different types of injuries. These miRNAs can serve as sensitive biomarkers for several diseases, including cancer (Schöler *et al*., 2010). The surprisingly high stability of miRNAs in the serum was explained by the observation that miRNAs could be part of exosome particles (Mitchell *et al*., 2008). The functional cycles of MIRNAs that have been examined in this study are MIRNA 133, 155, 185, and 217, which lead to VHL, VEGFA, JAK-2, BACH1 and CUL4B proteins with specific functions.

Maroto *et al* examined Cabozantinib, a targeted therapy used in the treatment of various types of cancer, including kidney cancer. This therapy operates by inhibiting the growth of new blood vessels that nourish the tumor and blocking specific signaling pathways that contribute to the progression of cancer. In the current study, the authors applied the results of Pablo Maroto regarding the impact of the drug Cabozantinib on Protein VEGFA and its inhibition of the angiogenic pathway (Maroto *et al*., 2022).

Roulin *et al* examined a combination with sorafenib, it instigates interference within the pathway PI3K/mTOR, ultimately fostering heightened apoptosis rates and controlling cancer cell proliferation. In the current study, the authors applied the results of Roulin regarding the impact of the drug sorafenib on the protein PI3K/mTOR inhibitor and its increasing Apoptosis pathway (Roulin *et al*., 2012).

Based on prior research on proteins, neoangiogenic activation under these conditions is accompanied by changes in the complex of molecular markers associated with VEGFR2 receptor signaling, the Akt/mTOR pathway, and PI3K/mTOR inhibitors (Ito *et al*., 2016; Roulin *et al*., 2011), which initiates the growth and proliferation of tumor cells (Cho, 2013; Spirina *et al*., 2017). VEGFR and VEGFA, a key activator of tumor angiogenesis, are discovered to be overexpressed in the majority of solid tumors (Apte *et al*., 2019). Several anti-angiogenic medications have been created to target either VEGFA or VEGFR in light of these facts (Li *et al*., 2019). These substances demonstrated anticancer activity by inhibiting JAK-2 and STAT-3 phosphorylation caused by COX-2. Immunohistochemistry was used to confirm this effect in colon tissue utilizing JAK2, p-JAK2, STAT3, and p-STAT3 targets (Rai *et al*., 2018). In breast cancer tumors as well as other tumor types,

BACH1 gene expression is inversely correlated with ETC gene expression (Lee *et al*., 2019). Frequently overexpressed in cancer, cullin 4B (CUL4B), which functions as a scaffold protein in CUL4B-RING ubiquitin ligase complexes (CRL4B), represses tumor suppressors through a variety of epigenetic processes (Wang *et al*., 2021). This study found that the presence of two proteins, VEGFA and BACH1, effectively predicts the effectiveness of the treatment since they may be treated with seven different medications.

In the drugs studies, the function of CABOZANTINIB, DOXORUBICIN, EVEROLIMUS, NELARABINE, SORAFENIB, SUNITINIB, and TEMSIROLIMUS drugs is specified in thyroid cancer (Elisei *et al*., 2013), RBMS2 Chemo sensitizes Breast Cancer Cells (Xu *et al*., 2022), inhibits breast cancer cell growth through PI3K/AKT/mTOR signaling pathway (Du *et al*., 2018), acute lymphoblastic leukemia in children (Kumamoto *et al*., 2020), liver cancer (Lin *et al*., 2020), hepatocellular cancer (Qi *et al*., 2020), endometrial cancer (Aghajanian *et al*., 2018), respectively. The medicine SUNITINIB which demonstrated the most significant outcomes in this investigation and displayed acceptable binding energy, molecular weight, site of action, and mutagenicity, can be extremely beneficial. However, it should be administered with greater caution due to its toxicity and its capacity to cross the blood-brain barrier. Earlier research has established the role of these proteins with various cycles in kidney cancer. In addition, it has been established how these medications work to treat tumors as well as occasionally kidney cancer. In this research, by examining the precise action of the drugs on the target proteins, we intended to achieve targeted treatments. Given the potential toxicity of these drugs, it is imperative to refrain from additional drug prescriptions without proper evaluation. In conclusion, we have attained satisfactory results through our efforts.

5. Conclusions

The drug SUNITINIB has yielded the best result, which has acceptable binding energy, molecular weight, site of action, and mutagenicity, which can be very useful, but due to its toxicity and crossing the blood-brain barrier, it should be prescribed more carefully. Also, the good response of two proteins 2IHC and 6Z13 with 7 drugs gives this prognosis that the doctor can be more flexible for treatment. It is a big step in the fight against KIRC tumor. Hopefully, these predictions will give acceptable answers in the laboratory and clinical trial phase and initiate targeted treatments in KIRC. Specialist doctors can treat patients with different severity of the disease with the least secondary complications. This is very important because of the traumatic nature of the treatments.

Author Contributions

Conceptualization, Saber.S. and S.F.; methodology, Saber.S. and S.F.; software, Saber.S., A.N., Sahel.S. ; validation, S.F., and Saber.S. ; formal analysis, A.N. and Saber.S. ; investigation, Sahel.S., N,G., F.G., N.M., and R.A; resources, A.N.; data curation, Saber.S.; writing—original draft preparation, Saber.S., Sahel.S., S.F. and A.N., writing—review and editing, Saber.S., N,G., F.G., N.M., R.A and S.F.; visualization, Saber.S. and S.F.; supervision, Saber.S. and S.F.; project administration, Saber.S.; funding acquisition, Saber.S. All authors have read and agreed to the published version of the manuscript.

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Abbreviations
2D	**Two-Dimensional**
3D	Three-Dimensional
Bach1	BTB and CNC homology 1
Bcl-2	B-cell lymphoma 2
CUL4B	Cullin ubiquitin ligases 4B
HB	HYDROGEN BONDS
HI	HYDROPHOBIC INTERACTIONS
JAKs	Janus-activated kinases
KICH	kidney chromophobe
KIRC	kidney renal clear cell carcinoma
KIRP	kidney renal papillary cell carcinoma
miRNA	MicroRNA
OB	OTHER BONDS
PLIP	Protein ligand Interaction Profiler
RCC	Renal cell carcinoma
VEGF	Vascular Endothelial Growth Factor
VEGFA	Vascular Endothelial Growth Factor A
VHL	Von Hippel Lindau

Declaration of competing interest
The authors declare that there is no conflict of interests.

Data Availability
Data will be made available on request.

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