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Anticancer Activity of *Calotropis procera* by Molecular Docking Analysis

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Abstract

Cyclin-dependent kinase 2 (CDK2) is a protein synthesized by Calotropis procera. It is known to be responsible for causing cancer in the human body. Studies have presented the significance of the phytochemicals to inhibit the CDK2 protein. Adsorption, distribution, metabolism, and excretion (Swiss ADME) and PubChem were the pharmacological tools which were used to check molecular weight, which should be less than 500D, acceptors less than 10, and donors less than 5, Log P less than 5 and gastrointestinal (GI) absorption should be high and Blood Bank Barrier (BBB) is also identified. If the drug-likeness is absent and has 2 violations then there will be no drug discovery. Using PubChem, the ligands were searched and the Simplified molecular-input line-entry system (SMILES) was copied and pasted to the list of smiles box in Swiss ADME and ran the process. From that, we come to know the molecular weight, acceptors, donors log p values, etc. By using a protein data bank (PDB) we got a PDB ID for CDK2 protein, i.e., 5NEV. Later 3d ligand binding site and active site prediction for protein was identified. The total ligands used here are 5 and they are calotropagenin, anthraquinone, flavonoid (ternatin), coumarin, and cannogenin. Then all the ligands were cleaned using Marvin's sketch. Energy minimization of protein was done by using the Swiss PDB viewer and then the PyRx tool (Visualization tool) was used to check the least binding affinity of the ligands. From the 5 ligands one which has the least binding affinity is taken for the docking process. Here, the ligand which is having least binding affinity is anthraquinone which is bound to the CDK2 protein. And later the docking visualization was performed using Biovia Discovery Studio and AutoDock Tools-1.5.6.

Keywords: Cyclin-dependent kinase 2 protein, anthraquinone, Lipinski rule, Ramachandran plot, anticancer activity

INTRODUCTION

Among Indian medicinal plants, *Calotropis procera* belongs to the milkweed family, and parts of the plant extracts are traditionally used for the relief of pain and inflammation. It mainly helps in anticancer, anti-inflammatory, and many other diseases, and experimental studies have reported that aqueous flower and latex extracts of *Calotropis procera* relieve pain, fever, and inflammation. Pharmacokinetics depends on absorption, distribution, metabolism, and excretion (ADME) parameters. Generally, bioavailability is estimated as per Lipinski's 'rule of five' in which it was known that better bioavailable of a compound depends upon <5 hydrogen bond donors, <10 hydrogen bond acceptors, <500 Daltons molecular mass, <5 a partition coefficient log P-value and <10 rotatable bonds. Drug-likeness is based on experiments in which orally active compounds with a highly probable range of physicochemical properties can be used as oral drugs [1]. The Swiss ADME online tool is a valid alternative for experimental drug design from

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natural products or synthetic compounds. This tool helps find a narrow range of compounds for future experimental work on pharmacokinetics and bioavailability, leading to new drug development [2].

The 5NEV is a four-chain structure derived from Homo sapiens. It plays a crucial role in regulating the cell cycle and is essential for meiosis, but not necessary for mitosis. This protein initiates the duplication of centrosomes and DNA and manages the timing of entry into mitosis or meiosis by subsequently activating cyclin B/CDK1.

This study aimed to detect the bioavailability potential through absorption, distribution, metabolism, and excretion (ADME) of phytocompounds established in *Calotropis procera* plant parts. The computational prediction of pharmacokinetics, bioavailability, drug-likeness, and medical chemistry friendliness was performed using the Swiss ADME online tool to identify lead phytocompounds for the prevention of pain and inflammation [3].

Molecular docking plays a vital role in structural biology and computer-aided drug design. It involves predicting the main binding mode of a ligand to a protein with a known three-dimensional structure [4]. The main objective is to obtain a ligand-receptor complex with minimal binding free energy. Successful docking methods navigate high-dimensional spaces and use scoring functions to accurately rank candidate docking.

Docking is a powerful tool used in drug discovery to virtually screen large libraries of compounds, rank them, and determine how these compounds might inhibit a target. Molecular docking has three main objectives: predicting how a compound fits into a receptor site, screening many compounds to identify potential binders, and estimating how strongly they bind. For docking to be successful, it is necessary to accurately predict how a compound fits into the binding site of the receptor and identify the key interactions. When dealing with a large number of compounds, this method must be able to identify which compounds bind well and rank them correctly. This process relies on algorithms to search for possible conformations, and scoring functions to evaluate their energy states. Handling the flexibility of molecules and accurately describing their interaction energies are crucial for making docking a reliable tool for designing new drugs [6].

In this review, we explore the search algorithms and scoring functions predominantly employed in contemporary molecular docking methods for protein-ligand applications. We aimed to summarize the key topics and recent computational and methodological advancements in protein-ligand docking. Additionally, we examined approaches that incorporate protein flexibility and strategies designed to enhance binding affinity prediction within the framework of docking-based investigations.

Cyclin-dependent kinases (CDKs), depicted in Figure 1, are part of the crucial Ser/Thr protein kinase subfamily and have been extensively studied for their vital roles in the cell division cycle, transcription, differentiation, neuronal function, and apoptosis. When CDK2 becomes active, it drives cells to advance through the cell cycle. This activation is crucial for several important cellular functions, including regulating the cell cycle, replicating DNA, responding to DNA damage, and controlling cell death pathways [7, 8]. Therefore, targeting CDK2 could re-emerge as a therapeutic avenue for restraining cancer cell proliferation.

LIGANDS

Anthraquinone groups are highly photoreactive when exposed to long UV light in the range of 340-360 nm. Flavonoids are a significant group of natural compounds that function as secondary plant metabolites with polyphenolic structures. They are commonly found in fruits, vegetables, and beverages. The term "coumarins" is derived from "*coumarou*," the common name for the Tonka bean, where coumarin was initially isolated in 1820. Coumarins belong to the benzopyrone family of compounds [9].

Calotropagenin, also known as a cardiac glycoside, has therapeutic use as an inhibitor of Na^+/K^+-ATPase to regulate heart contractions. The 3D structures of the phytochemicals are shown in Figure 2.

It is also known as peruvoside. It is a cardiac glycoside that can cause heart failure [10].

PHYTOCHEMICALS

Anticancer Activity

Cancer is a serious disease that is spreading worldwide. It is the scariest, as it can relapse even with the regimen or medication. Plants such as *Calotropis procera* are considered toxic, as they have a wide variety of medicinal properties used against ailments such as chronic inflammatory disease, in the treatment of cold, cough, pathological hemorrhoids, and ulcers. In the realm of traditional Indian medicine, the AK plant has been utilized to address various health issues such as ulcers, leprosy, tumors, and piles. The root extract of *C. procera* demonstrated potent cytotoxic effects on COLO 320 tumor cells. Cancer continues to be a leading cause of morbidity and mortality globally, with breast cancer being the most common and prevalent cancer among women. Cardiac glycosides have been shown to trigger apoptosis in diverse cancer cell types, leading to tumor suppression in humans [11, 12].

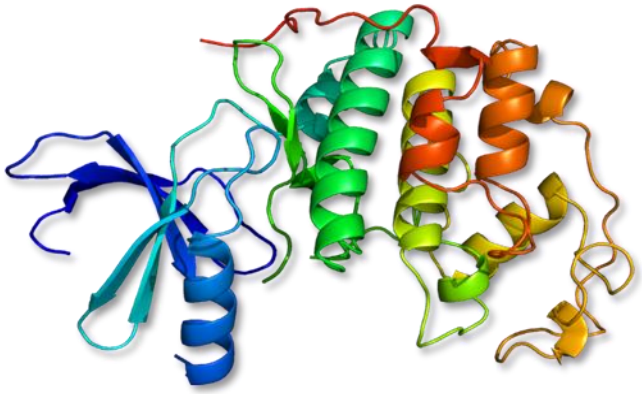

Figure 1. 3D structure of cyclin-dependent kinase (CDK2) protein.

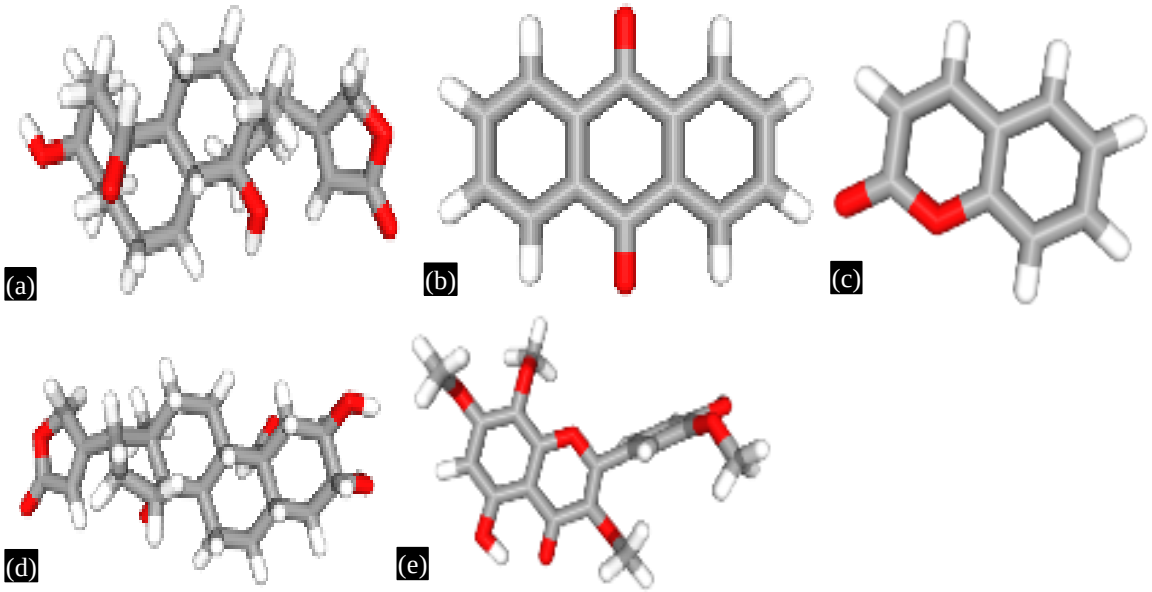

Figure 2. 3D structures of phytocompounds, (a) 3D structure of cannogenin, (b) 3D structure of anthraquinone, (c) 3D structure of coumarin, (d) 3D structure of calotropagenin, (e) 3D structure of flavonoid.

METHODOLOGY

Protein Retrieval

The protein receptor was obtained from the RCSB PDB and was used to obtain the structure by downloading the PDB format. The chosen receptor was subjected to energy minimization using the Swiss PDB viewer (SPDBV).

Ligand Preparation

The structure data file (SDF) of the chosen ligands, calotropagenin, anthraquinone, flavonoid (ternatin), coumarin, and cannogenin, were obtained from PubChem. SDF-to-PDB file conversion was accomplished using an SDF-to-PDB file converter. Ligand cleaning was performed using Marvin's sketch.

Analysis of Drug-likeness of the Ligand

Swiss ADME is considered a conventional drug discovery tool. All properties of the chosen ligands were investigated using this tool. It includes Lipinski filter analysis, pharmacokinetic behavior, that is, blood-brain barrier, and gastrointestinal (GI) absorption.

Docking Evaluation

Molecular docking of the anthraquinone ligand with the 5NEV protein receptor was achieved using PyRx and AutoDock tools-1.5.6. The docking process allowed us to interpret the active binding site of a specific ligand. The entire receptor was placed within the grid box to produce a blind docking with each ligand. The results generated nine outcomes consisting of binding affinity and RMSD values.

RESULT AND DISCUSSION

The Ramachandran plot shows a statistical distribution. The Ramachandran plot displays the main chain conformation angles (Ψ) of the polypeptide chain of CDK2 protein (Figure 3). Total amino acid residues were 236, and 10 amino acid residues are not shown. Absence of glycine and proline amino acid residues.

The chart uses color-coding for clarity:

- Black, dark gray, gray, and light gray indicate highly preferred conformations, with delta values ≥ -2.
- White with a black grid signifies the preferred conformations, with delta values between -2 and -4.
- White with a gray grid denotes questionable conformations, with Delta values <-4.
- Highly preferred observations are shown as green crosses: 227 (96.186%).
- Preferred observations are shown as brown triangles: 8 (3.390%).
- Questionable observations are shown as red circles: 1 (0.424%).
  Not Shown: 10
  Total: 236

Lipinski Filter Analysis

Drug-likeness was analyzed using the Lipinski filter (Table 1). It provides information on pharmacokinetic properties, molecular weight, number of hydrogen bond donors and acceptors, and molecular refractivity.

DOCKING RESULTS

PyRx

The results generated using PyRx consisted of nine values for each ligand. Anthraquinone exhibited the lowest binding affinity. These results were obtained using blind docking. The binding affinity results helped to predict the possible active sites for a ligand. Figure 4 shows the active site prediction of the protein.

AutoDock Analysis

The docking results were obtained using AutoDock tools 1.5.6. The RMSD table includes ten outcome values for each ligand. From the results of autodocking, the binding sites in the protein for ligand interactions were identified. Anthraquinone has the lowest binding affinity (i.e.-7.3). Figures 5 and 6 show ligand anthraquinone bound to receptor protein cdk2 in 3D and 2D, respectively.

DOCKING VISUALIZATION

It is an interaction between the natively can't and residue of ACE (Angiotensin-converting enzyme) which can be used to guide docking experiments in the search of selective natural component. Visualization is done by looking for the score function of your program uses.

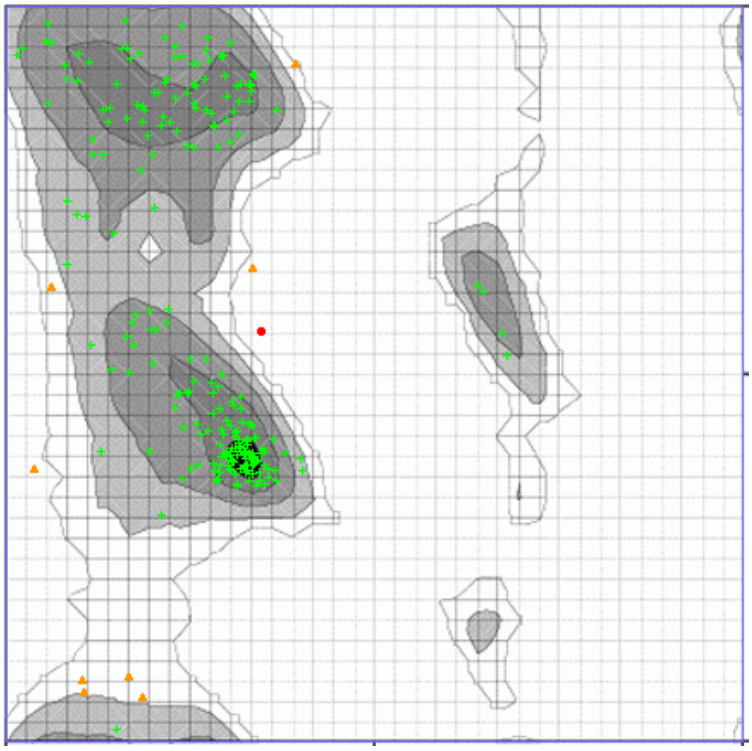

Figure 3. Ramachandran plot analysis of CDK2 protein.

Table 1. Phytochemical components.

Phytochemicals	Flavonoid	Coumarin	Cannogenin	Anthraquinone	Calotropagenin
Molecular weight	374.3g/mol	146.14g/mol	388.5g/mol	208.21g/mol	404.5g/mol
No. of hydrogen atom donor	2	0	2	0	3
No. of hydrogen atom acceptor	8	2	5	2	6
Molecular refractivity	97.3	42.48	104.96	59.75	134.03
Blood-Brain Barrier	No	Yes	No	Yes	No
GI Absorption	High	High	High	High	High
Permeability Glycoprotein Substrate	No	No	Yes	No	Yes
Log S (scale insoluble) <-10<poorly <-6 moderately <-4<soluble<-2<very<0<highly	-4.60 (moderately soluble)	-2.29 (soluble)	-3.13 (soluble)	-3.82 (soluble)	-3.60 (soluble)

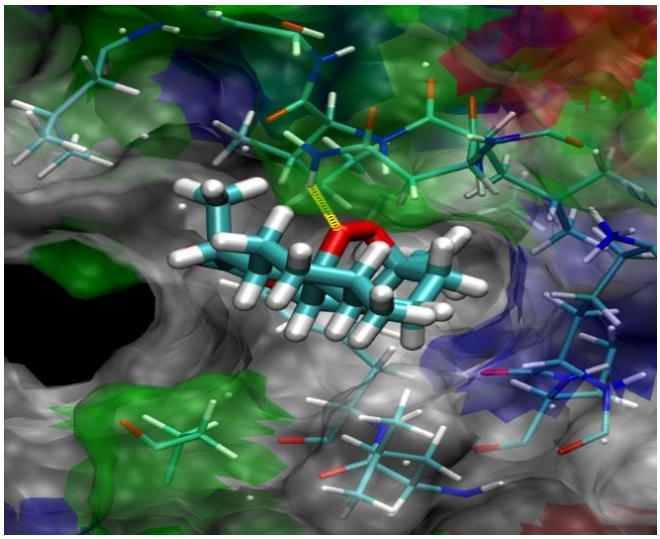

Figure 4. Active site prediction of the protein.

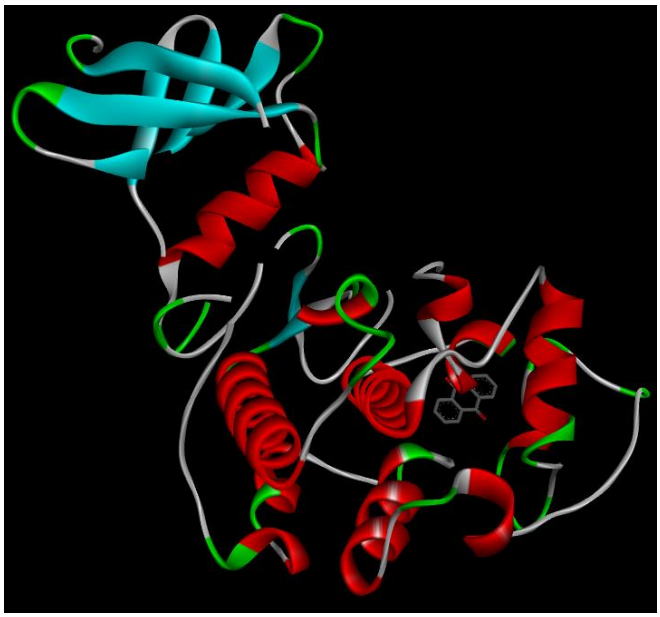

Figure 5. Ligand anthraquinone is bound to the receptor protein CDK2.

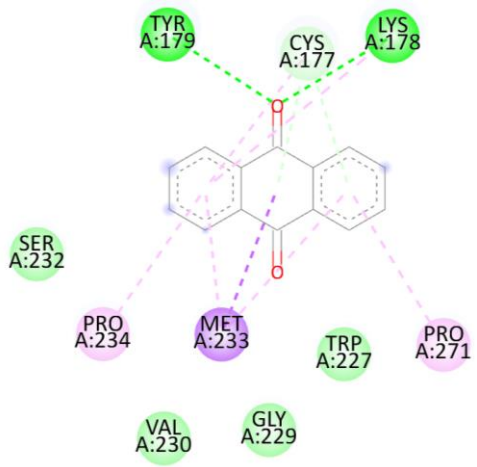

Figure 6. 2D diagram of anthraquinone protein interaction.

CONCLUSION

Based on docking studies, anthraquinone, coumarin, cannogenin, flavonoid, and calotropagenin have shown favorable outcomes. Through Swiss ADME analysis, it was observed that all five ligands showed high GI absorption, whereas calotropagenin and cannogenin have BBB permeability.

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