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Original Article

Sesquiterpene Lactones as Potential Cyclin B1/CDK1 Complex Inhibitors

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ABSTRACT

Cancer is second most common cause of death globally. Uncontrolled regulation of cell cycle may cause various cancerous anomalies. **Objective:** To Identify of Sesquiterpene Lactones (SLs) as inhibitors of Cyclin B1 (CB1) and Cyclin Dependent Kinase 1 (CDK1) complex. **Methods:** Checkpoints proteins (CDK1/CB1) of G2/M phase have been assessed with three SLs (ilicol, eucalyptone and ascleposide E) through molecular docking study. AutoDock Vina (ADV), PyMol version-2.5.2 and BIOVIA Discovery Studio 2021 was used for the visualization of docking analysis. **Results:** Outcomes of the current investigations reveal that ascleposide E exhibit the highest binding affinity of -7.1 kcal/mol (with inhibition constant of 5.9 µM) with CDK1 and CB1. Both potential complexes have shown good hydrogen bond interactions. Drug likeness of selected drug candidates were validated by ADMET analysis and Lipinski's rule of 5. **Conclusions:** Present study concluded that Ascleposide E have greater inhibition potential against CB1/CDK1 protein complex by making hydrogen and hydrophobic interactions. Moreover, this selected compound showed favorable drug likeness profiling. To validate the inhibitory activity of Ascleposide E to greater extent, further *in vitro* investigations are recommended to develop this compound into novel G2/M phase inhibitors.

INTRODUCTION

Cancer stands second in causing the greatest number of deaths worldwide. In 2023, 1.9 million new cancer cases and 609,360 cancer deaths are projected to occur in the United States [1]. Cancerous anomalies may develop because of cell cycle dysregulation. [2]. Cell cycle is a highly regulated event in normal physiological conditions and its transition through each phase is being controlled by checkpoints or regulatory proteins i.e., cyclins and CDKs. Whereas, transition through G2/M phase is regulated by stable complex of CDK1 and CB1 proteins [3, 4]. Cancer cells compromise this checkpoint and undergo uncontrolled proliferation. Targeted inhibition of CDK1 and CB1 can induce the cell cycle arrest and halt the uncontrolled proliferation of cancer cells as reported by many studies [5]. Therefore, by having deep insight of regulatory processes of cell cycle, advance and effective strategies can bring forth novel agents as anti-cancer drugs. Prevailing anti-cancer drugs are cytotoxic and expensive however, plant-based compounds are much safer, cost effective. Therefore, these compounds have been exploited for their potential anticancer activities [6]. SLs, firstly extracted from Asteraceae plants, are the broad class of plant based bioactive compounds that possess many biological activities [7, 8]. SLs reported to have significant *in vitro* and *in vivo* activity in multiple cancers and experimental models, respectively [9]. Methodology

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of MD has been used as an effective tool for predicting and synthesizing compounds for their biological activities and pharmacological interactions in conventional drug discovery [10]. The current study investigate interaction of three compounds of SLs against CDK1 and CB1. The present study will emphasize on investigating therapeutic potential of selected compounds against G2/M cell cycle arrest.

METHODS

The process of MD has its extensive use in obtaining ideal conformations and binding energies of protein-ligand complex [11, 12]. ADV was used for the docking analysis as it offers accurate analysis of interactions occurring between protein and ligand. For obtaining PDBQT format of both ligands and proteins PyMol version-2.5.2 and MGL tools 1.5.7 were used. BIOVIA Discovery Studio 2021 was used for the visualization of docking results as well as analyzing different interactions between protein and ligand [13]. Three-dimensional structure of CB1 (PDB ID: 2B9R) and CDK1 (PDB ID: 4YC6) were retrieved from RCSB PDB database. Several modifications including addition of Kollman charges and polar hydrogen and removal of water molecules were made in structure of proteins in ADV for further processing. After these edits, protein structures were saved in PDBQT formats. Three-dimensional structures of ilicol, eucalyptone and ascleposide E were obtained from PubChem database in SDF formats and converted into PDBQT formats so that ADV can better compute it for further analysis. Docking was performed using ADV. Grid box formation was carried out by 40×40×40 dimensions and 0.375 Å spacing. The ideal conformation was selected on the base of lowest binding affinity. Further interactions between stable complex were visualized by BIOVIA Discovery Studio 2021. Absorption, Distribution, Metabolism, Excretion and Toxicity (ADMET) analysis was carried out for the estimation of pharmacokinetic properties of understudy ligands. For this study, pkCSM (online server of database) was used for predicting ADMET profile and drug likeness [14, 15]. SMILES of proposed ligands were retrieved from PubChem, entered into pkCSM, and analyzed

RESULTS

SLs are widespread in various food and medicinal plants. A plethora of studies highlights their several therapeutic potentials such as anti-cancer, anti-inflammatory, anti-tumor and anti-oxidant activities [7, 16]. Three novel ligands including ascleposide E, ilicol and eucalyptone were selected. Their chemical structures are shown in Figure S1. The binding mechanism of protein and ligand is requisite for exploring promising new drug candidates. So, for extensive understanding of protein-ligand interactions, MD is widely used as a tool for the prediction of binding modes and scores of proteins-ligand interactions [17, 18]. The ligands were docked against CB1 and CDK1 of G2/M checkpoints. Their crystal structures can be seen in Figure S2 (a) and (b). Among all ascleposide E exhibit highest binding score of -7.1 kcal/mol with inhibition constant (k_i) of 5.9 µM with both proteins (Table 1).

Table 1: Binding energies, inhibition constant of CDK1 and CB1 in complex with different SLs

Ligands (SLs)	CDK1		CB1	
	Binding Energy (kcal/mol)	Inhibition Constant (µM)	Binding Energy (kcal/mol)	Inhibition Constant (µM)
Ilicol	-6.4	19.4	-6.8	9.8
Eucalyptone	-7.0	7.0	-5.7	63.7
Ascleposide E	-7.1	5.9	-7.1	5.9

Discovery studio visualizer was used for the visualization of interactions of protein-ligand complexes. The docking of eucalyptone shows binding affinity to the Chain A and B of CB1 with six hydrogen bonds including PHE131 (2.02Å), GLY134 (2.66Å), ARG68 (2.55 Å) and ARG68 (2.87Å) and one hydrophobic interaction with GLY132 (4.53Å) (Figure 1).

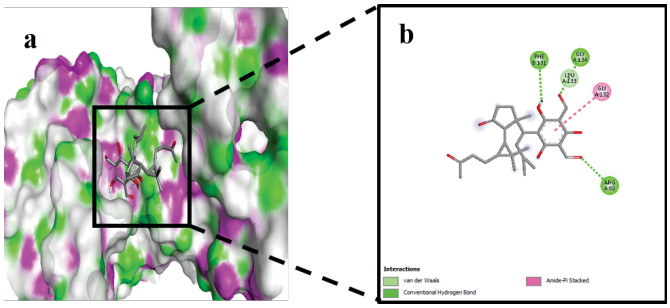

Figure 1: Eucalyptone docked with binding pocket of CB1 (a), 2D diagram of CB1 and Eucalyptone complex (b)

With CDK1, eucalyptone forms hydrogen bond with GLN49 (3.37Å), GLN49 (1.87Å), GLN132 (2.14Å), LYS88 (2.78Å), LYS88 (2.73Å) and ILE6 (2.00Å) and one hydrophobic interaction with TYR8 (3.82Å) (Figure 2).

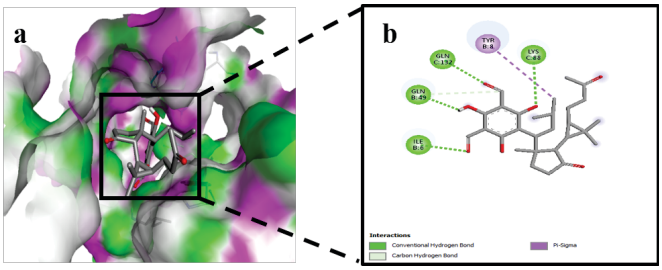

Figure 2: Eucalyptone docked with binding pocket of CDK1 (a), 2D diagram of CDK1 and Eucalyptone complex (b)

Results obtained by docking of ilicol with CB1 showed single hydrogen bond with ALA22 with a bond distance of 2.11Å and hydrophobic interaction with VAL63, PRO138 and MET167 with bond distances of 5.48Å, 4.87Å and 5.19Å, respectively (Figure 3).

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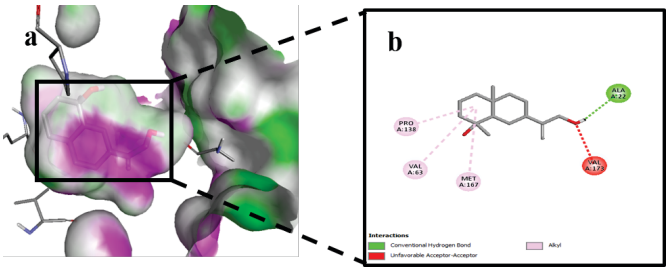

Figure 3: Ilicol docked with binding pocket of CB1 (a), 2D diagram of CB1 and Ilicol complex (b)

However, ilicol shares three hydrogen bonds with TH161 (3.70Å), LYS88 (2.50Å) and LYS88 (2.75Å) and hydrophobic interaction with TRP168 (5.26Å), VAL165 (4.27Å) and VAL165 (5.46Å) amino acid residues of CDK1 (Figure 4).

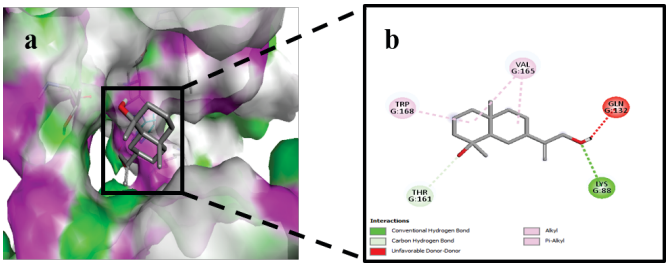

Figure 4: Ilicol docked with binding pocket of CDK1 (a), 2D diagram of CDK1 and Ilicol complex (b)

Ascleposide E docked with CB1 shows significant binding with chain B. The interactions of this complex are characterized by six hydrogen bonds with ASN72 (2.00Å), GLY134 (2.55Å), LEU133 (2.79Å), ARG68 (2.26Å), ARG68 (2.03Å) and ARG68 (2.98Å) residues (Figure 5).

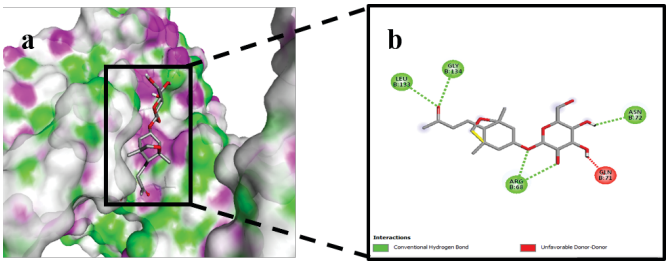

Figure 5: Ascleposide E docked with binding pocket of CB1 (a), 2D diagram of CB1 and Ascleposide E complex (b)

Ascleposide E forms five hydrogen bonds with GLN49 (2.39Å), ARG20 (2.48Å), ARG20 (2.59Å), GLN132 (2.12Å) and LYS88 (2.06Å) residues of CDK1 (Figure 6). These results suggest that studied compounds might have anti-cancer potential.

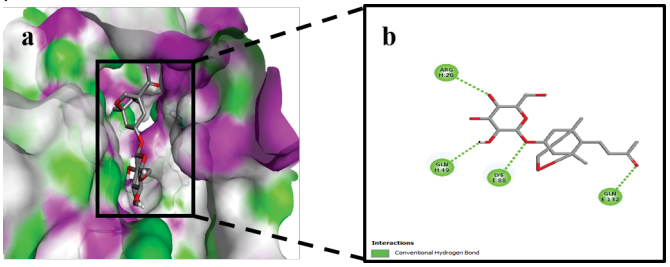

Figure 6: Ascleposide E docked with binding pocket of CDK1 (a), 2D diagram of CDK1 and Ascleposide E complex (b)

Both ascleposide E and ilicol follow Lipinski's rule of 5, whereas eucalyptone showed violation in this case (log P > 5) (Table S1). Pharmacokinetics assessment of studied drug candidates are shown in table 2, which reveals that eucalyptone, was found to be inhibitor of P-glycoprotein I/II and CYP3A4 substrate. In addition to that, water solubility lies in the range of -2.9-3.9 mol/L. All the compounds showed good intestinal absorption as well as clearance of drug from the body. Toxicity analysis showed that all the drug candidates have no hepatotoxicity and AMES toxicity.

Table 2: ADMET analysis of studied ligands

ADMET	Parameters	Ilicol	Eucalyptone	Ascleposide E
Absorbance	Water solubility (log S) mol/L	-3.194	-3.977	-2.938
	P-glycoprotein I/II inhibitor	No	Yes	No
	Intestinal absorption%	93.228	91.219	48.811
Distribution	log VDss (L/Kg)	0.306	0.068	-0.21
Metabolism	CYP2D6 inhibitor	No	No	No
	CYP3A4 inhibitor	No	No	No
	CYP2D6 substrate	No	No	No
	CYP3A4 substrate	No	Yes	No
Excretion	Renal OCT2 substrate	No	No	No
	Total clearance (Log ml/min/kg)	1.129	0.24	1.142
Toxicity	AMES	No	No	No
	Hepatotoxicity	No	No	No
	Max. tolerable dose (log mg/kg/day)	0.15	-0.203	0.08

DISCUSSION

The main objective of current study is to investigate anti-cancer potential of novel plant-based compounds as well as inhibitors of CDK1/CB1 complex by using molecular docking and bioinformatics tools. Our findings revealed that all three selected drug candidates interact strongly with the residues of CDK1 and CB1 indicating it as potential inhibitors. However, ascleposide E has shown highest binding affinity of -7.1 kcal/mol. A plethora of reports has shown anti-cancer potential of many SLs by inhibiting CDK1/CB1 complex in various cancer cell lines, thus arresting cell cycle. In a recent investigation, another SL 1β, 2α-epoxytagitinin C (a derivative of tagitinin C) was reported to induce cell cycle arrest at the G2/M phase by inhibiting the CDK1/CB1 complex in HPB-ALL cells thus demonstrating it as potential anti-leukemia agent [19]. Similarly, treatment of burkitt lymphoma cells and breast cancer cells with japonicone A belonging to same SL group resulted in inhibition of CB1 and CDK1 which leads to the arrest in the G2/M phase of cell cycle [20]. In addition to that, various other SLs have been shown to inhibit CB1/CDK1 complex in breast cancer [21], human lung adenocarcinoma and human prostate cancer [22]. These findings may suggest our studied compounds as potential

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CDK1 inhibitors as it belongs to same SLs compound group. Moreover, these compounds have shown promising drug likeness profiling and drug bioavailability. A drug candidate should have good absorption and fast excretion from the body to make it an orally active drug. For this purpose, drug likeness must be predicted via Lipinski's rule of 5. This rule stated that, any ligand is considered to be drug-like if it is likely to have no more than 5 hydrogen bond donors, no more than 10 hydrogen bond acceptors, molecular mass less than 500 Da and log P should be less than 5 [23]. Only eucalyptone (log P > 5) showed violation in this case while ilicol and ascleposide E are suitable as a drug. For drug discovery, prediction of pharmacokinetic properties for drug candidate is requisite criterion. ADMET profile analysis would be helpful in drug's bioavailability [24]. Once the drug is intake orally, it must be absorbed in small intestine, which is an essential attribute for determining its bioavailability [25]. Absorbance of any drug candidate should not be less than 30% [26]. So, all of our selected drug has absorbance of more than 30%. In addition to that, water solubility (log S) of all drug compounds is greater than -5 mol/L which indicate their efficacy to be soluble in water at 25˚C [15]. The drug that inhibit P-glycoprotein I/II transporters may cause disturbances in the pharmacokinetics interactions of other drugs [27]. However, our results reveal that eucalyptone is P-glycoprotein I/II inhibitor while the rest of the drug molecules can be suggested as a potent adjuvant agent for arresting cell cycle at G2/M phase. Uniform distribution (VDss) of a drug in the blood plasma is an essential factor. The range value of VDss is -0.5 – 0.45. If its value for a drug is higher than 0.45 or less than -0.5, then it shows much distribution of drug in plasma instead of tissues and less diffusion of drug across cell membranes, respectively [28]. Current study shows all drug candidates to be ideal in terms of uniform distribution in blood plasma. Drug metabolizing enzymes also affect the drug's pharmacokinetic properties. Any change in the Cytochrome P450 (detoxification enzyme present mainly in liver) may interfere with the pharmacokinetics interactions of drugs which are metabolized by these enzymes. This enzyme exists in two isoforms namely cytochrome P2D6 and cytochrome P3A4 [29, 30]. According to our results, only eucalyptone inhibits these enzymes. However, other ligands have no role in inhibition of the enzyme. Organic cation transporter 2 (OCT2) is a transporter of protein which play significant role in clearance of drug. Substrate of this protein may interact with its inhibitor (OCT2 inhibitor) and cause harmful reactions [15]. Current study reveals that all drug candidates are not substrate of OCT2. AMES toxicity shows if the compound is mutagenic or not. Similarly, hepatotoxicity determines the potential of drug to cause liver injury [29]. Our results showed that none of the compound is hepatotoxic or AMES toxic.

CONCLUSIONS

In this study, we investigated three SLs against CB1/CDK1 protein to arrest cell cycle at G2/M phase. By molecular docking study, we found that ascleposide E have greater inhibition potential against CB1/CDK1 protein complex by making hydrogen and hydrophobic interactions. Moreover, this selected compound showed favorable drug likeness profiling, which make it a potent inhibitor leading to cell cycle arrest at G2/M phase. To validate the inhibitory activity of ascleposide E to greater extent, further *in vitro* and *in vivo* investigations are recommended to develop this compound into novel G2/M phase inhibitors.

Authors Contribution

Conceptualization: MK
Methodology: AZ, ZY
Formal analysis: AZ, ZY, SG
Writing-review and editing: MK, MA

All authors have read and agreed to the published version of the manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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