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RESEARCH ARTICLES

In silico analysis of plumbagin against cyclin-dependent kinases receptor

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Abstract

Cancer is an abnormal growth of cells which could migrate from its place of origin to other parts of the body. It is one of the most serious disease on which widespread research work has been going on. Computer aided drug designing has helped in the identification of potential leads that can be used for the development of a drug. Plumbagin is a naphthoquinone derivative from *Plumbago zeylanica* roots which possess strong anticancer properties. Plumbagin has been shown to produce inhibitory effects on multiple cancer-signaling proteins. However, binding mechanism and molecular interactions have not been elucidated yet for most of the target proteins. In this investigation, an attempt was done to explore the binding mechanism of plumbagin against cyclin-dependent kinases (CDK) receptor using molecular docking. The least binding energy of plumbagin with CDK6 was found to be − 6.18 kcal/mol. The molecular simulation suggests that plumbagin has potential binding affinities with CDK6 and its interactions with CDK6 was quite stable during the whole period of simulation run. It was also found that plumbagin obeys Lipinski's Rule of 5 and has drug likeness proved by ADMET analysis. As plumbagin is a natural compound, it has reduced side effects and these results would be useful for cancer treatment.

Keywords Cancer · Plumbagin · CDK6 · Molecular docking · Dynamics

Introduction

In-silico method is extensively used in pharmacology and chemistry to find out the information at the molecular level. Computational results help in explaining the molecular interactions and suggest probable mechanisms that are involved in the process. Structure-based virtual screening and post-screening analysis methods are an important step in hit identification and development of a lead molecule. Nowadays cancer is one of the major causes of death globally. Changes in lifestyle, and consumption of junk foods have immensely contributed to the burgeoning cancer cases (Jemal et al. 2011). The onset of cancer has been linked with various mutations in signalling proteins, molecular biomarkers and important genes associated with normal cell proliferation. A range of signal transduction pathways are involved in cell growth regulation. Therefore, any minute changes in these pathways might lead to tumor pathogenesis. The most significant reversible mechanism to inhibit the specific protein activity in a signaling pathway is phosphorylation. This elementary process is achieved through different protein kinases activity. In recent years, significant development has been done to understand the cancer progression and treatment. The therapeutic arsenal of tumor treatment includes radiation therapy, chemotherapy, surgery, immunotherapy and hormonal therapy and their possible combinations as well. However, these treatment methods possess harmful side effects, therefore there is a demand for finding more influential and lesser toxic anticancer agents.

Medicinal plants are widely used for the treatment of various diseases across the world since ages in Ayurveda and indigenous cultures of different countries, therefore research groups often screen a library of medicinal plant compounds for the development of new drugs. They contain a wide range of phytocompounds which include alkaloids, flavonoids, sterols, glycosides, etc. Medicinal plants can suppress proliferation of cancerous cells due to the presence of various cancers inhibiting compounds like taxols, phytosterols, naphthoquinones, etc. Over 3000 plants have been

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recognized which showed anticancer properties (Roy et al. 2017). Additionally, significance of plant-derived drugs for cancer treatment is growing at a rate of 10–40% (Molassiotis et al. 2006). Approximately 60% of the medications that are available for cancer are attributed to plant sources (Roy et al. 2018). Utilization of plant secondary metabolites having anticancer properties is a preferred choice for cancer therapy (Grover et al. 2012).

Plumbago zeylanica is a medicinal plant, also known as "Chitrak", belongs to the family '*Plumbaginaceae*'. Roots of this plant are commonly used for the treatment of piles, anasarca, diarrhea, cancer, rheumatism and leprosy (Roy and Bharadvaja 2017a, b). This plant possesses antibacterial, anti-fungal, anti-cancer, anti-plasmodial, anti-tumour, stimulatory activity, anti-inflammatory, anti-hyperglycemic, central nervous system and hepatoprotective activity (Roy and Bharadvaja 2017a, b, 2018a, b). Medicinal properties of this plant are due to the presence of a compound i.e. plumbagin. Experimental studies suggest that due to its anti-inflammatory and anti-tumor effect, plumbagin may show protection against various cancers such as oral, colon, blood, breast cancer, ovarian cancer, leukemia, prostate cancer, etc. (Jamal et al. 2014; Tripathi et al. 2019). Inhibitory role of plumbagin is due to alterations of various signaling pathways which play a vital role in cancer cell proliferation, survival, invasion, and metastasis (Jamal et al. 2014). The chemopreventive effect of plumbagin on in vivo cancer model was also studied (Sakpakdeejaroen et al. 2019).

Current situation demands more effective therapies for cancer. Various molecular targets that are responsible for cancer have been recognized, one of the potential targets to inhibit various diseases is cyclin-dependent kinases (CDKs) which is a serine/threonine kinases and extensively studied due to their vital roles in cell cycle division, neuronal functions, transcription, differentiation and apoptosis (Li et al. 2015). These kinases get activated only in the presence of a specific cyclin partner. CDK6 has an important function in the regulation of cell cycle and up-regulation of CDK6 is associated with the development of numerous cancers like colon, breast, bladder, pancreatic and oral cancers (Liu et al. 2015; Kawasaki et al. 2016; Li et al. 2017). Expression of CDK6 is more in cancer cells and low in normal cells. These studies showed that CDK6 has a particular oncogenic function in cancer treatment that can be useful for designing potential anti-cancer drugs having low toxicity (Tadesse et al. 2015). CDK6 has a vital role in the proliferation of cells and cancer. Therefore, development of inhibitors for CDK6 is required to treat cancer. In this study we performed in silico analysis comprising molecular docking of plumbagin against uncoupled CDK6 protein, validation of Lipinski's Rule of 5, ADMET analysis and molecular dynamic simulation to analyse the substantial outcome in support of the proposed idea that plumbagin could inhibit CDK6 function in cell cycle and its mechanism of action was also elucidated.

Materials and methods

Preparation of protein and ligand

Three-dimensional structure of CDK6 (PDB: 3NUP) was retrieved from Protein Data Bank (PDB). The structure of plumbagin (ligand) (CID: 10,205) was retrieved from NCBI—PubChem.

Active site prediction

Understanding the reaction of catalytic residues is essential for correct function of an enzyme. Information regarding active site of CDK6 was reported earlier by Zhang et al. (2018) and was used in this study.

Preparation of receptors

To prepare receptors for docking analysis, co-crystallized water molecules, nonpolar hydrogens, lone pairs, small molecules and non-standard residues were omitted, and polar hydrogens and Gasteiger charges were added. Hydrogens were added to reasonably generate protonation states at physiological pH.

Molecular docking

AutoDock 4.2 is a commonly utilized tool for docking studies, it has the efficiency to predict fast and precise ligand to targets binding confirmations. It is a grid-based process which is used for rapid estimation of binding energy of test confirmations. Docking protocol included the processing of ligand by removing coordinates and water molecules from its PDB file. For conformational searching, Lamarckian genetic algorithm was used. Main residues were placed in a grid of 60 Å × 60 Å × 60 Å (x, y, z). Finally, protein–ligand interaction was observed in discovery studios (2019).

Molecular dynamic simulation (MDS)

MDS of the target CDK6 protein with Plumbagin was performed using non-commercial Desmond version 2019.4 (D. E. Shaw Research, New York, 2019; Schrödinger). For the description of water molecules, TIP3P model was used. The neutralization of each complex was done using a salt concentration of 0.15 M. The standard relaxation protocol and other standard settings given in the Desmond module package were used. For production runs of 1 ns, 5 ps time steps with NPgT ensemble at 300 K were deployed for simulating each

system prepared. For each simulation, Root mean square deviation (RMSD), Potential energy, and Root mean square fluctuations (RMSF) values were recorded and observed for analysis of the stability of the complex.

Drug likeliness and bioavailability

Any compound needs to follow Lipinski's rule of five to be formulated as a drug. SWISSADME online tool is used to analyse drug likeness and bioavailability. It provides LogP, mass, hydrogen bond acceptor, hydrogen bond donor and molar refractivity of a compound as well as other pharmacokinetic parameters and bioavailability.

Results and discussion

Molecular docking

CDK6 has a bilobal fold with 326 amino acid residues in which 1–100 amino acid residues comprises the N-terminal lobe which has 5 antiparallel beta sheet strands and the alpha C-helix. C terminal lobe is quite large which contains residues 101–326. A hinge connects the both lobular folds. The location of the ATP binding site is at the lobal interface and the hinge forms one of its edges. A highly conserved DFG-motif and the activation loop (or T loop) comprises residues 163–189 are present in the C-terminal lobe part of the ATP binding site. Glu61, Lys43 and Asp163 are the key catalytic residues which are not properly positioned in inactive CDK6 due to obstruction by T loop. On binding with cyclin partner, Glu61 is positioned near to Lys43 and Asp163 which helps in the proper orientation of ATP for the nucleophilic attack and also Thr177 is exposed after binding with cyclin partner which is phosphorylated by CDK activating kinases (Schulze-Gahmen and Kim 2002). Palbociclib (PD0332991) has been shown to inhibit CDK6 by binding to the ATP binding site. The major interactions observed in case of PD0332991 was hydrogen bond with Val101, Asp102 and Asp163 and the substituents in Palbociclib were blocking the important Phe98 residue as well (Davies et al. 2002).

Molecular docking score of plumbagin with CDK6 was observed to be − 6.18 kcal/mol. For comparison of docking score, inhibitor that is already present in the PDB structure of 3nup was used. 4-(Pyrazol-4-yl)-pyrimidine was the inhibitor and docking score for this complex was -6.48 kcal/mol. So, plumbagin is quite close to the already established inhibitor of CDK6. Plumbagin in the docked complex formed H-bond with Glu-99 and Val101, hydrophobic interactions with Ile19, Lys43, His-100 and Asp163, and showed interactions with other important residues like Phe98, Val27 and others (Fig. 1). Favorable interactions with all these residues clearly demonstrate that Plumbagin occupies the ATP binding site as Palbociclib and moreover its interactions

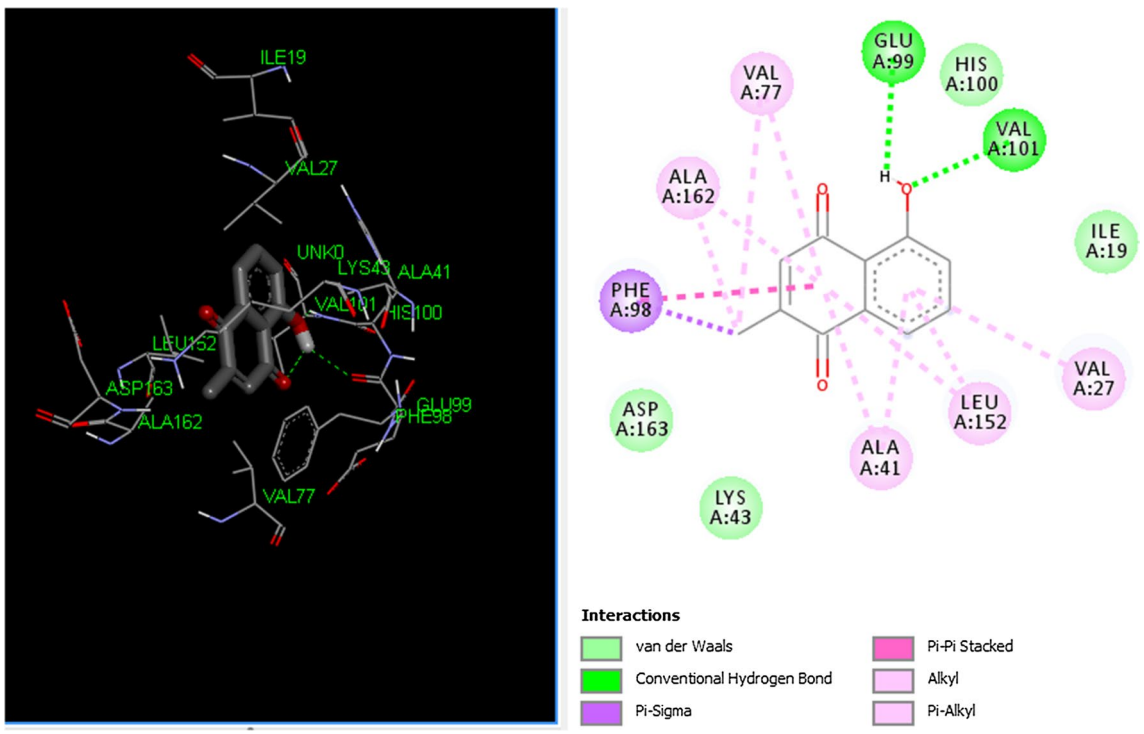

Fig. 1 Docking of CDK6 with Plumbagin

with catalytic residues Lys43 and Asp163 could also help in the inhibition of CDK6 which could be further explored. Hydrophobic interaction with His-100 might give plumbagin a selectivity towards CDK4/6 as it has been reported that interaction with His100 could lead to 26-fold selectivity against CDK 4/6 (Cho et al. 2010).

CDK has a significant role in the regulation of cell cycle and administers cellular transitions from growth phases to linked with DNA replication and mitosis (Bertoli et al. 2013). Due to the differential expression of CDKs, checkpoint control could be affected which would result in neoplastic conversion. A report suggested an enhanced expression of CDK6 in acute lymphoblastic leukemia and its function in pathogenesis (Aleem and Arceci 2015). A study reported unusual proliferation in acute lymphoblastic leukemia cells due to enhanced CDK6 expression and they suggested that CDK6 protein inhibition can serve as a significant therapeutic strategy (Agirre et al. 2009). Cho et al. (2010) reported that ligand (4-(pyrazol-4-yl)-pyrimidines) interact with Lys 43 and Val 101 residues which indicates that these residues are important binding site residues. Jayaraman and Jamil (2014) also found that ligands interact with the Ile 19, Lys 43, Gly 25, Glu 99, Phe 98, Val 101, His 100, Asp 104, Asp 102, Lys 147, Asp 145, Gln 149, Asp 163 residues of CDK6 receptor. Zhang et al. (2018) reported that the binding efficiency of flavonoids against CDK6 were primarily through hydrogen bond and van der waal interactions with Ile19, Val27, Ala41, Asp163, Phe98, Glu61, Gln103, and Leu152 residues. In our docking study also, we found that plumbagin interacts with these residues through H-bonds. This shows its importance as a potential inhibitor of the target molecule.

Molecular dynamic simulation

Throughout the simulation period, prominent hydrogen bonds were formed with residue VAL101 and ASP163 direct as well as through water bridges. Besides this, ILE19, GLN149 and ASN150 also interact through water bridges. Hydrophobic interactions were shown by ILE19, VAL27, ALA41, PHE98, LEU152 and ALA162 (Figs. 2, 3). In our study, VAL101 and ASP163 form hydrogen bonds with plumbagin for most of the simulation time which is similar to the previously conducted study by Hernandez Maganhi et al. (2017) where palbociclib was used as a potential CDK6 inhibitor. The binding residues obtained were mostly similar to our study. The average RMSD value for the plumbagin-CDK6 complex was 1.65 A which was approximately similar to the study done by Hernandez Maganhi et al. (2017). In another study, flavonoids were investigated for their inhibitory effects against CDK6-cyclin D complex (Zhang et al. 2018). They also obtained similar molecular dynamics simulations results where binding residues, interactions and RMSD scores were well in conjunction with our results (Zhang et al. 2018). These major interactions in the binding pocket and the active site of these molecules clearly show the relevance of simulations results. Hydrogen bonding with important residues in the active site of these molecules shows the relevance of results and the potential effect of plumbagin as an inhibitor of these cancer targets.

Drug likeliness and ADMET prediction

Lipinski's Rule of Five is utilized to check the drug likeness that is to assess if a compound shows the desired pharmacological and physiological properties needed to make it orally active drug candidate for humans. According to Lipinski's rule of 5, molecular weight of a drug should be < 500 Da, should have log P (ratio of drug concentration in octanol: water) value < 5, number of H bond donor < 5, number of H bond acceptor < 10 and Molar refractivity should be in between 40–130. SWISSADME analysis of plumbagin reported a molecular weight of 188 Da, log P value of 1.72 and Molar refractivity of 51.07 (Fig. 4). The compound has one hydrogen bond donor and three hydrogen bond acceptors. Therefore, plumbagin follows all the parameters of Lipinski's Rule of 5 and possess drug likeliness.

SWISSADME also predicted pharmacokinetic parameters of plumbagin which includes high GI absorption, blood brain barrier permeability, substrate or inhibitor of CYP1A2, no P-glycoprotein substrate, and not a inhibitor of CYP2C19/CYP2C9/CYP2D6/CYP3A4. As plumbagin is not a substrate of these Cytochrome P450 proteins, its metabolism would be lesser which could be further explored in in vivo *assays.* When the count of rotatable bonds is increased, the compound exhibits more flexibility and is better suitable for efficient ligand protein interaction in a précised binding pocket (Sliwoski et al. 2014). More TPSA (Topological Polar Surface Area) indicates hydrophilic compound and less TPSA represent hydrophobic compound. Ideally, TPSA should be less than or equal to 140 Å (Wipf et al. 2015). Lesser TPSA value is related to more drug distribution and easy permeation of the cell membrane barrier as lower value corresponds to more hydrophobicity. TPSA of plumbagin is 54.37 Å which is in the lower range of PSA values thus its distribution across the cell membrane would be good. All of these observations clearly accomplished that plumbagin can efficiently act as a drug.

Conclusion

Present study showed that inhibition of CDK6 a potential cancer target can be done by a natural compound plumbagin and its mechanism of action has been elucidated. Plumbagin was binding in the ATP binding site and showed interactions

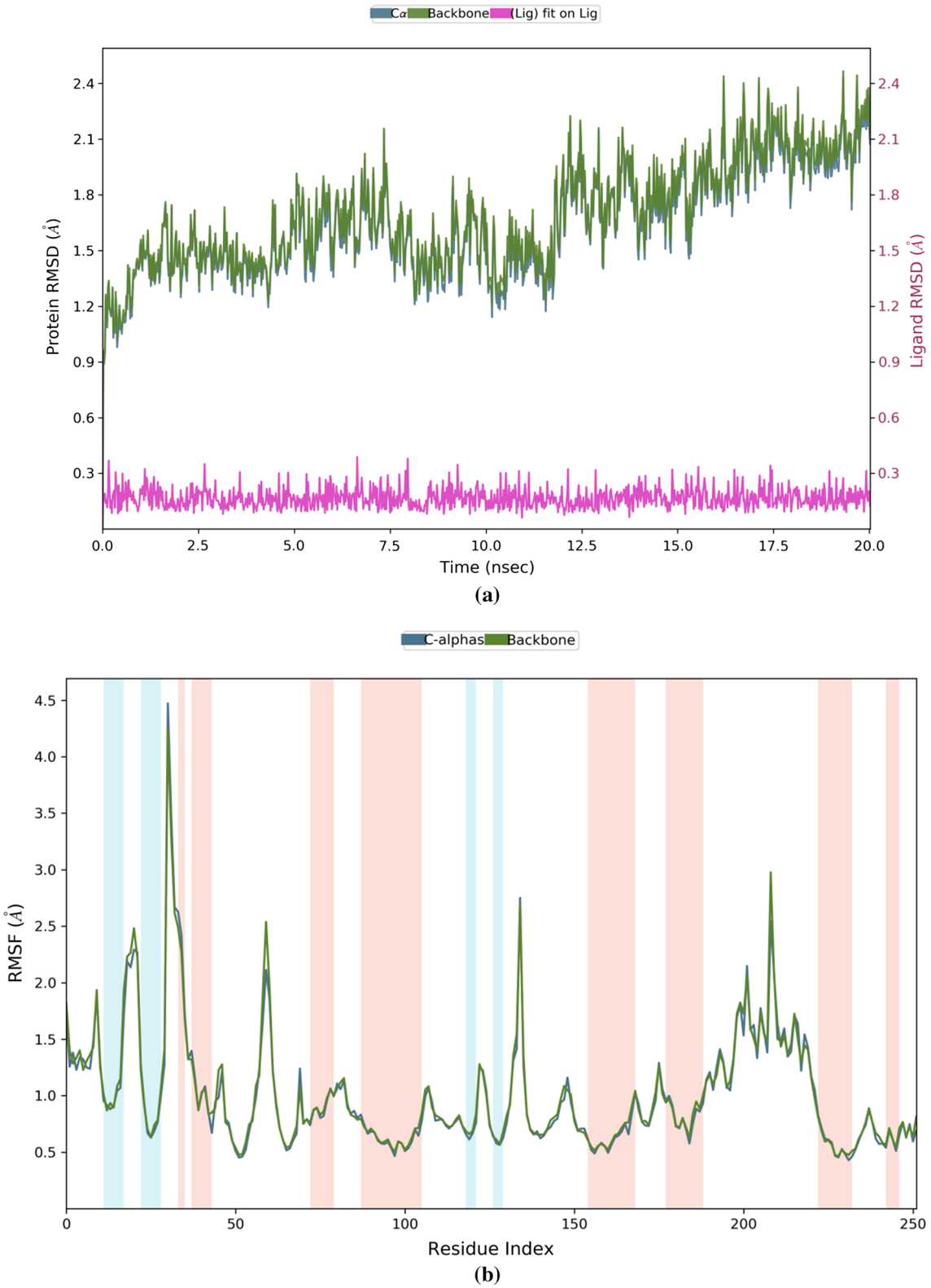

Fig. 2 (**a** RMSD plot for CDK6, **b** RMSF plot for CDK6 with secondary structural features

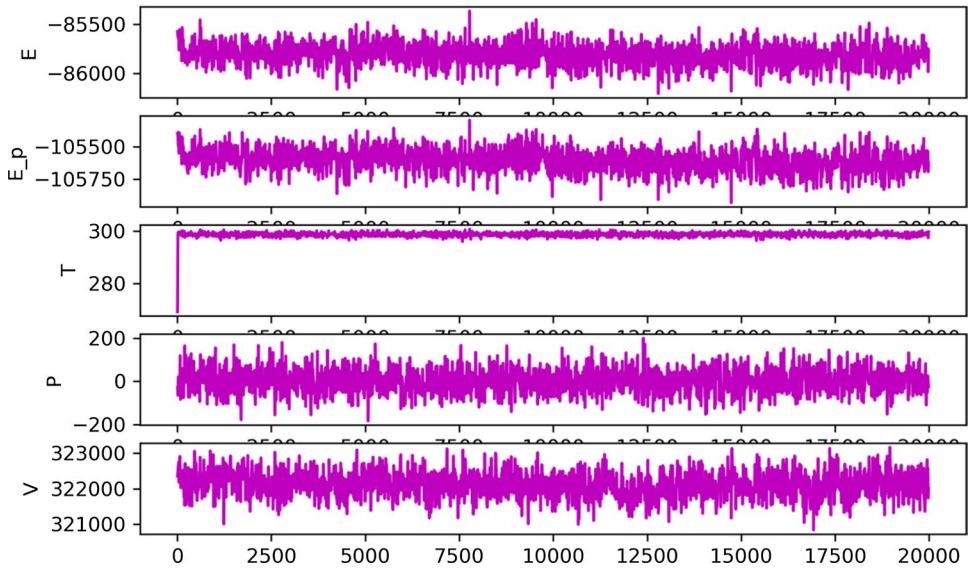

Fig. 3 Simulation quality analysis for CDK6

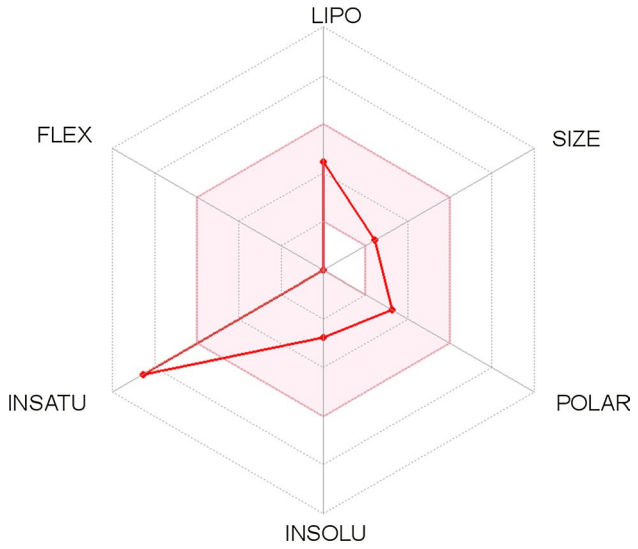

Fig. 4 Radar plot for Plumbagin after SWISSADME analysis

with important catalytic residues of CDK6. MD results confirm the stability of the docked complex and putatively predicted the inhibition of CDK6 active site. Plumbagin also obeys essential parameters to act as drug particularly follows Lipinski's Rule of 5. Present study explains the mechanism of action of plumbagin and it also suggest that it could act as a potential inhibitor of CDK6. Further evaluation of pharmacokinetics and pharmacodynamics of plumbagin can also be done.

Compliance with ethical standards

Conflict of interest Author declare no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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