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RESEARCH ARTICLE

Molecular Dynamics Mechanisms of the Inhibitory Effects of Abemaciclib, Hymenialdisine, and Indirubin on CDK-6

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Abstract: ***Background*:** Cyclin-Dependent Kinases-6 (CDK-6) is a serine/threonine protein kinase with regular activity in the cell cycle. Some inhibitors, such as abemaciclib, hymenialdisine, and indirubin, cause cell arrest by decreasing its activity.

***Objectives*:** The purpose of this study was to evaluate the Molecular Dynamic (MD) effects of abemaciclib, hymenialdisine, and indirubin on the structure of CDK-6.

***Methods*:** The PDB file of CDK-6 was obtained from the Protein Data Bank (http://www.rcsb.org). After the simulation of CDK-6 in the Gromacs software, 200 stages of molecular docking were run on CDK-6 in the presence of the inhibitors using AutoDock 4.2. The simulation of CDK-6 in the presence of inhibitors was performed after docking.

***Results*:** Abemaciclib showed the greatest tendency to bind CDK-6 *via* binding 16 residues in the binding site with hydrogen bonds and hydrophobic bonding. CDK-6 docked to hymenialdisine and indirubin increased the Total Energy (TE) and decreased the radius of gyration (Rg). CDK-6 docked to hymenialdisine significantly decreased the coil secondary structure.

***Conclusion*:** CDK-6 is inhibited *via* high binding affinity to abemaciclib, hymenialdisine, and indirubin inhibitors and induces variation in the secondary structure and Rg in the CDK-6 docked to the three inhibitors. It seems that developing a drug with a binding tendency to CDK6 that is similar to those of abemaciclib, indirubin, and hymenialdisine can change the secondary structure of CDK6, possibly more potently, and can be used to develop anticancer drugs. However, additional studies are needed to confirm this argument.

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1. INTRODUCTION

Cell cycle regulation plays a crucial role in cell fate. The Cyclin-Dependent Kinases (CDKs), a class of serine/threonine protein kinases, regulate the cell cycle [1]. Cell cycle arrest, at any of the phases, *i.e.* G0/G1, S, G2 and mitosis, increases the likelihood of apoptosis and arrests cell growth and proliferation, which is essential for the control of cancer cells [2]. Cell cycle arrest provides specific agents that play a role in cell damage repair with further opportunities to evaluate cell performance and confirm cell health [3]. In the case of cell genome damage in cell arrest, the DNA repair agents will have more time to repair the genome. However, if the damage is extremely severe, and the cell is unable to repair itself, apoptosis and cell death will occur [4].

The G0/G1 cell phase is the longest and the most important phase of cell division, in which the initial cell health is evaluated. If the regulatory factors do not detect any disruption in the cell, cyclin D will increase, causing the phosphorylation of CDK4 and CDK6, which in turn leads to the phosphorylation and activation of the Rb regulatory protein and, therefore, the cell cycle will progress naturally. The Rb protein phosphorylation will provide the necessary agents for entry into the S phase [5]. CDK-6 plays the most important role in controlling and inhibiting cell growth at the beginning of the cell cycle and the G0 phase. Therefore, the inhibition of this protein can serve as the target of many anticancer drugs to prevent cell entry into the first growth and division phase [6].

The three chemical compounds, abemaciclib, hymenialdisine, and indirubin, are known to be potent inhibitors of CDKs, that can be used to treat many types of cancers [7]. Abemaciclib is a synthetic compound used in many cancers,

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especially breast cancer, to inhibit CDKs and induce apoptosis and cell death [8, 9]. Indirubin is an alkaloid compound derived from the fermentation of certain medicinal plants, that, by exerting an inhibitory effect on CDKs, can produce anti-cancer effects and induce cell apoptosis [10, 11]. Hymenialdisine, which is derived from sea sponges, can produce inhibitory effects on CDKs [12]. In fact, abemaciclib, hymenialdisine, and indirubin can inhibit the cell at different stages of its growth by inhibiting the activity of CDKs. Meanwhile, the study on the effects of molecular dynamics of these compounds on CDKs can be useful to develop more potent inhibitors. This study aimed to investigate the molecular dynamic effects of these inhibitors on the CDK6 molecule under simulated conditions and to examine the changes induced by these dynamic effects on the structure and properties of this cell cycle regulating molecule.

2. MATERIALS AND METHODS

2.1. PDB Files Preparation

CDK-6 PDB file (ID: 5L2T) was obtained from the protein data bank server (www.rcsb.org) and optimized with the Arguslab software. Then, the antioxidant files of abemaciclib (CID:370), hymenialdisine (CID: 72281), and indirubin (CID:10281) were obtained from the Pubchem server which were optimized and converted to PDB files by the Arguslab software.

2.2. Simulation and Molecular Dynamics (MD) of CDK-6

Using the Gromacs version 4.5.4, studies on the molecular dynamics simulation of the CDK-6 were first performed in pure water and then in a G43A1 force field to reach balance under with changes in temperature and pressure. A 140 μM concentration was prepared by adding calculated values of Na and Cl**.** The SPC216 model was used in this study [12]. Then, the PDB file output was used as a molecular docking input to simulate complexes.

2.3. Molecular Docking

Molecular docking of abemaciclib, hymenialdisine, and indirubin was performed on CDK-6 to find the best binding sites for the ligand-receptor and to determine the most stable free energy state of the ligand-receptor. In this study, a grid box of 60×120×72 nm^3 (x×y×z) was created for the protein after the production of PDBQ and PDBQT for the abemaciclib, hymenialdisine, and indirubin molecules as ligands and for the CDK-6 molecule as the receptor. We used the *autogrid4 –p n.gpf –l n.gle* Linux command to produce the n.gle text file. After 200 runs of molecular docking on ligands, we used the Genetic Algorithm and Lamarckian GA parameters. The *autodock4–p n.dpf–l n.dlg* linux command was used to produce the n.dlg text file. The data obtained from the n.dlg file was analyzed [13, 14].

In this study, we used the LigPlot plus v.2.1 software to specify the number of hydrophobic bonds and hydrogen bonds between CDK-6 with abemaciclib, hymenialdisine, and **i**ndirubin. The type and number of amino acids present in the binding site were determined [15].

2.4. Simulation and MD Studies of CDK-6 and Inhibitors

At the last stage of molecular dynamics simulation, the CDK-6 protein complex was formed with abemaciclib, hymenialdisine, and indirubin ligands in 140 μM water and salt in accordance with the above method. As studied before, the paths involved in the simulation were used to analyze the structural parameters of the complex. The results of the simulation of the CDK-6 molecule alone and its complexes with each of these ligands were comparatively analyzed using the Grapher 10 software [16, 17].

The temperature was set at 300K for all the simulations and the time of the simulation was 10 nanoseconds (ns). In this study, docking and dynamic simulations were performed using a 64-bit system with Intel (R) Core (TM) i7 CPU Server.

2.5. Statistical Methods

The data were analyzed using SPSS version 22 (Chicago, IL, USA). The paired-sample t-test was performed to analyze molecular dynamics. $P<0.05$ was considered the significance level.

3. RESULTS

Table **1** shows the Binding Energy (BE), Final Intermolecular Energy (FIE), Estimated Inhibition Constant (EIC),

Table 1. Molecular interactions of abemaciclib, hymenialdisine, and indirubin with CDK-6.

Ligand- Receptor	BE kcal/mol	FIE kcal/mol	EIC	Interaction Bonds	
				Hydrogen Bonds	Hydrophobic Bonds
CDK-6 Abemaciclib	-8.3	-10.09	820.54 (nM)	Arg60, Glu61, Phe164	Val41, Arg140, Val142, Gly165, Val164, Leu68, Leu166, Lys43, Asp163, Val45, Phe98, Arg44, Leu96
CDK-6 Hymenialdisine	-6.25	-6.85	26.03 (μM)	Glu21, Lys43, Asp163	Asn150, Gln149, Gly20, Ile19, Lys29, Asp104, Leu152
CDK-6 Indirubin	-8.33	-9.22	787.54 (nM)	Arg60, Val142, Gly165	Glu61, Val64, Leu68, Arg140, Val141, Phe164, Leu166

Abbreviations: BE; Estimated Free Energy of binding (kcal/mol), FIE; final intermolecular energy (kcal/mol), EIC; estimated inhibition constant (μM).

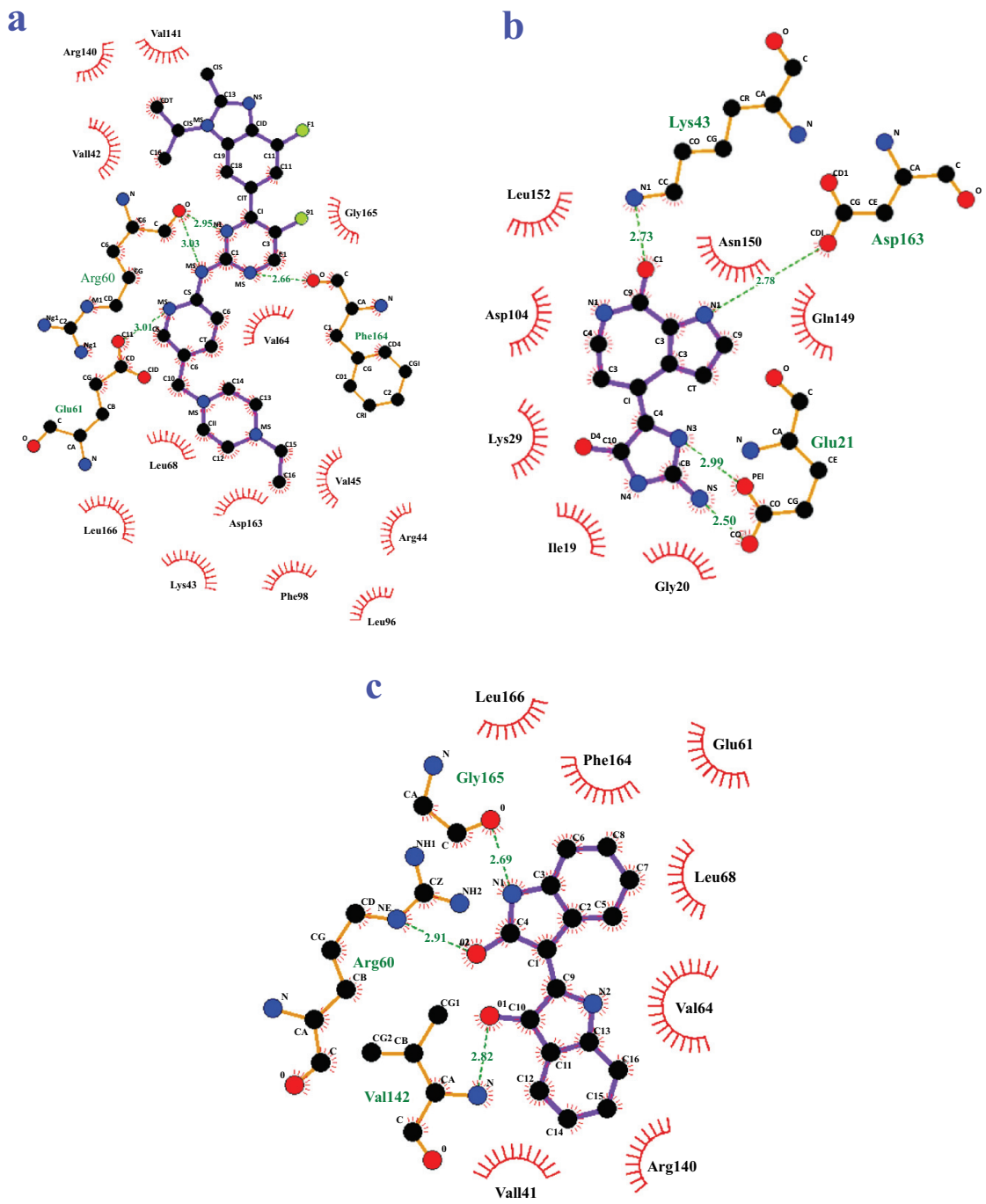

Fig. (1). The analysis of protein–ligand interactions in abemaciclib (**A**), hymenialdisine (**B**), and indirubin (**C**).

and the number of interaction bonds, such as hydrogen bond and hydrophobic bonding in CDK-6 in complex with abemaciclib, hymenialdisine, and indirubin.

The interaction bonds (hydrogen bond and hydrophobic bonding) between abemaciclib, hymenialdisine, and indirubin and CDK-6 residues are illustrated in Fig. (**1**).

The Root Mean-Square Deviation (RMSD) values for the simulation of CDK-6 alone and its complexes with abemaciclib (blue), hymenialdisine (red) and indirubin (green) at 10 ns of the simulation are illustrated in Fig. (**2**).

Fig. (**3**) shows the amount of Total Energy (TE) in the simulation of CDK-6 alone and its complexes with abemaciclib, hymenialdisine, and indirubin at 10 ns of the simulation.

Fig. (**4**) shows the value of the radius of gyration (Rg) in the simulation of CDK-6 alone and its complexes with abemaciclib (blue), hymenialdisine (red) and indirubin (green) at 10 ns of the simulation.

Molecular dynamics simulation parameters, such as TE, RG, and RMSD, of CDK-6 alone and its complexes with

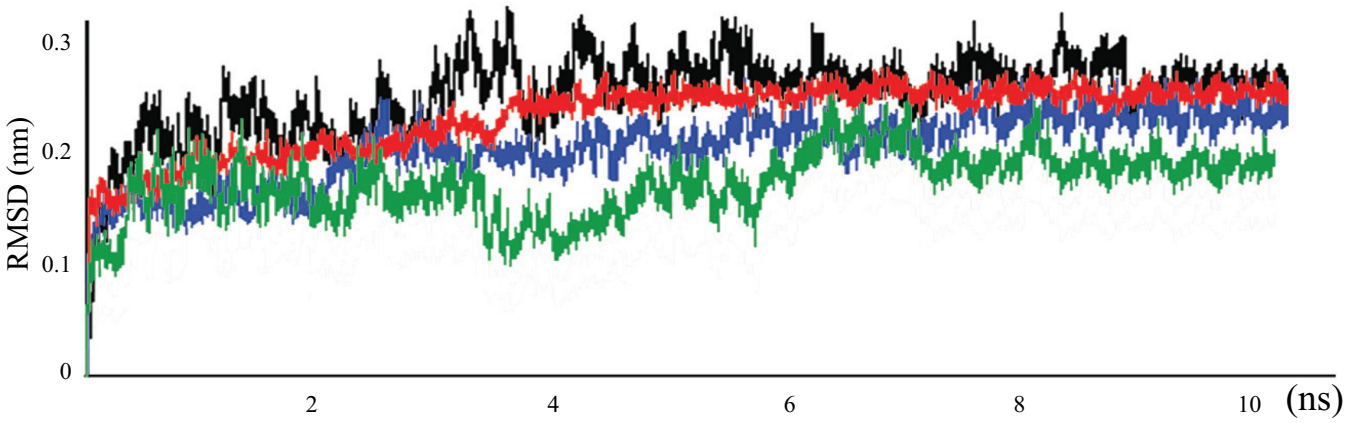

Fig. (2). The root mean-square deviation values of CDK-6 alone (black) and its complexes with abemaciclib (blue), hymenialdisine (red) and indirubin (green). Statistical analysis was done by the independent-samples t-test. Each point represents mean±SD.

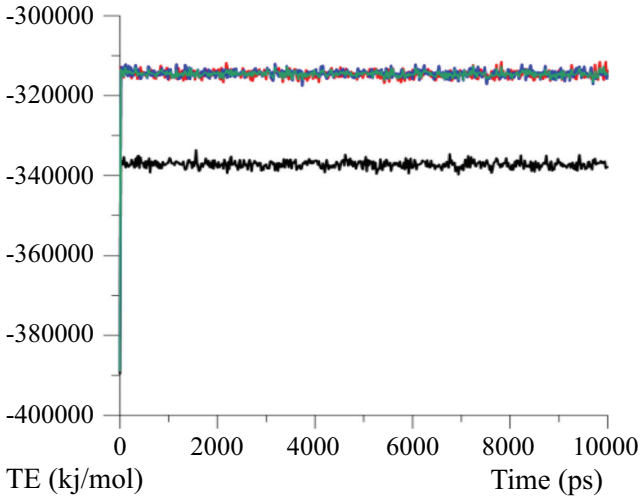

Fig. (3). Total Energy (TE) of CDK-6 alone (black) and its complexes with abemaciclib (blue), hymenialdisine (red) and indirubin (green). Statistical analysis was done by the independent-samples t-test. Each point represents mean±SD.

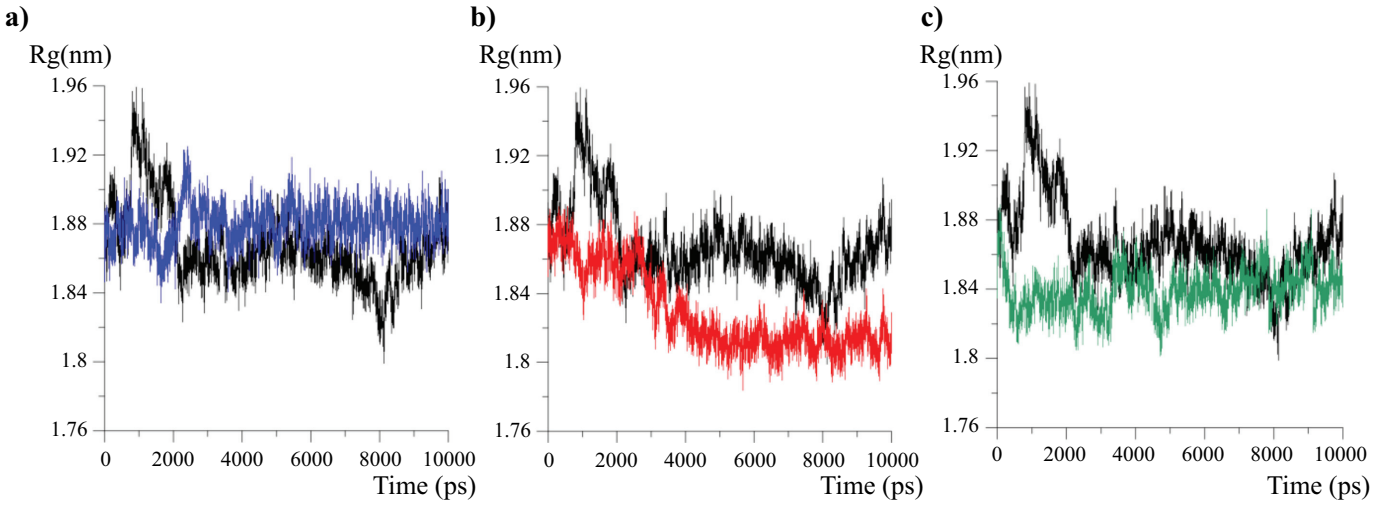

Fig. (4). Radius of gyration (Rg) of CDK-6 alone (black) and its complexes with abemaciclib (blue), hymenialdisine (red) and indirubin (green). Statistical analysis was done by the independent-samples t-test. Each point represents mean±SD.

Table 2. Molecular dynamics simulation parameters of CDK-6 complexes with inhibitors.

Simulation Parameters / Inhibitors	TE	RG	RMSD
CDK6	-337336(2502)	1.87(0.02)	**0.23(0.03)**
CDK6-Abemiciclib	-314797(3438)*	1.88(0.01)*	**0.18(0.03)***
CDK6-Hymenialdisine	-314594(3441)*	1.83(0.02)*	**0.19(0.03)***
CDK6-Indirubin	-313994(3328)*	1.84(0.01)*	**0.17(0.02)***

TE; Total Energy, RG; Radius of gyration, RMSD; Root-Mean-Square Deviation. Statistical analysis was done by the independent-samples t-test. Each point represents mean±SD; *P < 0.001 compared with CDK-6.

Table 3. Variation in the secondary structure of CDK-6 in complex with ligands.

Secondary Structure Protein-Ligand	Coil %	B-Sheet %	B-Bridge %	Bend%	Turn %	A-Helix %	3-Helix %
CDK6	24.2	14.6	1.2	13.1	10.8	33.8	2.3
CDK6-Abemiciclib	24.1	14.2	1.2	14.1	11.6	32.8	2.0
CDK6- Hymenialdisine	23.2	14.9	1.2	14.0	10.9	33.3	2.5
CDK6- Indirubin	24.1	14.6	1.4	13.4	11.3	33.1	2.1

abemaciclib, hymenialdisine, and indirubin, at 10 ns of the simulation are shown in Table **2**.

Variation in the secondary structure of the CDK-6 protein, as a factor to predict CDK-6 activity in complex with abemaciclib, hymenialdisine, and indirubin at 10 ns of the simulation is shown in Table **3**.

4. DISCUSSION

This study shows that abemaciclib, hymenialdisine, and indirubin, as the three inhibitors of CDKs, can bind to CDK-6 with high tendency and can induce structural changes in this serine/threonine protein kinase, influencing the cell cycle. Abemaciclib is one of the potent CDK-6 inhibitors [18]. In this study, abemaciclib showed a higher tendency to bind CDK-6 than indirubin and hymenialdisine. It forms 3 hydrogen and 13 hydrophobic bonds with CDK-6 in the binding site. This compound, with lowest concentration (820.54 nM), releases 8.3 Kcal/mol (BE=-8.3 Kcal/mol) in the presence of CDK-6 (Fig. **1** & Table **1**). Abemaciclib is an oral, potent, small inhibiting molecule of CDK4 and CDK6 activity, which blocks retinoblastoma tumor suppressor protein phosphorylation and thereby prevents the progression of cancer through the cell cycle [19]. The sea sponge constituent, hymenialdisine, is a potent inhibitor of cyclin-dependent kinases, glycogen synthase kinase-3β and casein kinase 1 [20]. Hymenialdisine and indirubin bind to CDK-6 with 3 hydrogen and 7 hydrophobic bonds in different binding sites (Fig. **1**), but their binding tendency to CDK-6 differs. Indirubin binds to CDK-6 (BE=-8.33 Kcal/mol) with a higher tendency and lower concentration (787.54 nM) than hymenialdisine (BE=-6.25 Kcal/mol and concentration of 26.03 μM) (Table **1**). Not only abemaciclib, hymenialdisine and indirubin also bind to CDK-6 with high tendency but they also inhibit cell cycle by exerting an effect on CDKs and decreasing their activity [21]. The simulation of CDK-6 alone and its complexes with three CDK inhibitors stabled after 8 ns. The RMSD of all significantly decreased after simulation in the presence of the inhibitors (Table **2**), which helped to reduce the activity of CDK-6 [15, 22]. Abemaciclib causes the TE to increase from -337336±2502 (CDK-6 simulation in the absence of abemaciclib) to -314797±3438 (CDK-6 simulation in the absence of abemaciclib) significantly by binding to and inhibiting CDK-6. This increase in the TE is also significant for hymenialdisine and indirubin **(**Fig. **3** & Table **2**). This study shows that, although hymenialdisine showed a lower tendency to bind to CDK-6 than indirubin and abemaciclib, it decreases the Rg of CDK-6 strongly. Hymenialdisine and indirubin decrease the Rg at 10 ns of the simulation compared with the simulation in CDK-6 alone. Rg is a measure of the protein radius. For globular proteins, high Rg means low compaction and higher function of the protein structure. Decreased Rg in the binding of hymenialdisine and indirubin to CDK-6 decreases the availability of the active sites and its activity [23]. Variation in the secondary structure of CDK-6 increased when abemaciclib, hymenialdisine, and indirubin docked to CDK-6 (Table **3**). Decrease in the coil in all the complexes of CDK-6 with three inhibitors, especially hymenialdisine, and other variations in the α-Helix and β-Sheet structures show that these inhibitors affect the secondary structure of CDK-6.

CONCLUSION

CDK-6 is one of the most important serine/threonine protein kinases that strongly inhibits cell cycle after inhibitors, such as abemaciclib, hymenialdisine, and **i**ndirubin decrease its activity. This study showed that the high binding affinity of these inhibitors to CDK-6 led to the inhibition of the spontaneous reactions and decrease in the released free energy. These three compounds cause changes in the secondary structure of CDK6 that may play a role in

inhibiting it. Abemaciclib and indirubin docked to CDK6 at lower doses in comparison with hymenialdisine, while hymenialdisine decreases Rg in comparison with two other drugs and increases the secondary coil active structure. It seems that developing a drug with a binding tendency to CDK6 that is similar to those of abemaciclib, indirubin, and hymenialdisine can change the secondary structure of CDK6, possibly more potently, and can be used to develop anticancer drugs. However, additional studies are needed to confirm this argument.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No animals/humans were used for studies that are the basis of this research.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

CDK-6 PDB file (ID: 5L2T) was obtained from the protein data bank server (www.rcsb.org) and optimized with the Arguslab software. Then, the antioxidant files of abemaciclib (CID:370), hymenialdisine (CID: 72281), and indirubin (CID:10281) were obtained from the Pubchem server which were optimized and converted to PDB files by the Arguslab software.

FUNDING

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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