MOLECULAR DOCKING AND QSAR STUDIES ON CDK4 INHIBITORS USING IN SILICO TECHNIQUES

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ABSTRACT
Cyclin-dependent kinases are a small family of serine/threonine protein kinases which control the cell cycle progression. Literature survey revealed that CDKs, their regulators, and substrates are the targets of genetic alteration in many human cancers. The best characterized case of such alteration is the p16-CDK4, 6-cyclin D-retinoblastoma pathway found in more than half of all human cancers. Therefore, CDK4 is an attractive target for the development of a novel anticancer agent. Computer aided drug design strategy has gained much prominence due to the fast and efficient means of studying protein-ligand interactions. A molecular docking was performed using Molegro Virtual Docker 6.0 with the CDK4 protein and the selected compounds from literature as ligands. QSAR toxicity analysis has been performed using FAF Drugs ADME/tox filtering server. Considering the molecular properties of the ligands, higher inhibitory activity is associated with reduced molecular flexibility, as measured by lower polar surface area (TPSA), LogP, lower hydrogen bond counts, confirming the capability of the compounds for binding at the active site of the receptor.

Keywords: Cyclin-dependent kinases, D-type cyclins, Cyclin-dependent kinase inhibitors, Docking, Molegro Virtual Docker 6.0, QSAR

INTRODUCTION
The cyclin-dependent kinases (CDKs) are a small family of serine/threonine protein kinases and require cyclin subunits for activity. They (Cdks) play a key role in regulating cell cycle progression and govern cellular transitions from growth phases (G1 and G2) into phases associated with DNA replication (S) and mitosis (M)\cite{1,2}. G1-S phase progression requires phosphorylation of the retinoblastoma (Rb) protein by Cdks\cite{3,4} or Cdks\cite{5,6} in complex with their activating subunits, the D-type cyclins, D1, D2, or D3.\cite{7,8,9,10}

Hyperphosphorylation of Rb diminishes its ability to repress gene transcription through the E2F family of transcription factors and consequently allows synthesis of several genes, e.g., the protein products of which are necessary for DNA replication\cite{11,12,13,14,15,16}. Thus, the catalytic activity of Cdks

which involves targeting the major regulators of CDK activity\cite{16,17,18,19} and the other is direct strategy which involves inhibition of the catalytic activity of the CDK kinases. Literature survey revealed that abrogation of the G1 checkpoint or acceleration of the Cdks/CDK D pathway provides a distinct advantage to cancer cells for proliferation and survival. Thus cyclin D–dependent kinases have been considered for many years a prime target for cancer chemotherapy\cite{20,21,22}. Natural products have historically and continually been investigated for promising new leads in pharmacological development. The activities of bioactive compounds and the synergistic action shown by them with other drugs make them ideal in alternative cancer therapies. The efficacy of various inhibitors reported in literature (both natural and synthetic origin) against CDK targets have been studied using computer aided drug design strategies. Molecular docking is an application, wherein molecular modeling techniques are used to predict how a protein interacts with small molecules (ligand). The concept of docking is used in the study of various properties associated with protein-ligand interactions such as binding energy, geometry complementary, electron distribution, hydrogen bond donor acceptor properties, hydrophobicity and polarizability. Thus few compounds (both natural and synthetic origin) reported in literature as possible cdk-4 inhibitors are tested computationally by docking program Molegro, their binding affinities are evaluated and QSAR toxicity analysis has been performed using FAF Drugs ADME/tox filtering server in order to design novel compounds which can act as better CDK4 inhibitor.

MATERIALS AND METHODS
Preparation of protein structure
The crystal structure of the CDK4 protein (PDB ID: 2W9Z) has been obtained from RCSB Protein Data Bank (http://www.pdb.org). All water molecules were removed and hydrogen atoms were added to the target protein molecule on the final stage.
Preparation of ligand structures
All the compounds used for docking study were selected from the literature\textsuperscript{22-31}. Ligand structures were constructed using Chem Sketch Software (http://www.acdlabs.com), three dimensional optimizations were done and then saved in mol file. Using Hartree-Fock (HF) calculation method by Argus Lab 4.0.1 Software, geometry optimization of the ligands were performed.

Protein ligand interaction using Molegro Virtual Docker 6.0
Docking program, Molegro Virtual Docker was used in analysis with default parameters. CDK4 protein was docked against the obtained ten ligands using Molegro Virtual Docker 6.0. Docking of the protein - ligand complex was mainly targeted only on to the predicted active site. Docking simulations were performed by selecting "Mol Dock" as the docking engine. A spacing of 0.3 Å between the grid points was used, "Mol Dock" was chosen as the calculation type, and the Mol Dock Score [GRID] was used as the scoring function. At maximum 10 poses were allowed to be analyzed. After completion of docking, the docked protein (protein - ligand complex) was analyzed to investigate the type of interactions. The docking poses saved for each compound were ranked according to their dock score function. The pose having the highest dock score was selected for further analysis.

Ligand screening and QSAR studies
FAF-Drugs is an online absorption, distribution, metabolism, excretion and toxicity prediction tool used to calculate molecular descriptors for all the inhibitors taken for the docking analysis.

RESULTS AND DISCUSSION
In order to understand the interactions between the ligands and CDK4 protein and to explore their binding mode, docking study was performed using Molegro Virtual Docker of version 6.0. The crystal structure of the CDK4 protein (2W9Z) was derived from PDB and used as a target for docking simulation. The compounds selected from the literature were listed in Table 1. Ligands for the docking procedure were constructed using Chem Sketch and energy minimized using Argus Lab 4.0.1. The structures of the ligands obtained from the Chem Sketch were shown in Figure 1.

Docking Studies
The goal of protein-ligand docking is to predict the predominant binding model(s) of a ligand with a protein of known three dimensional structures. Crucial information concerning the orientation of the inhibitors in the binding pocket of the target protein was obtained from docking studies. Inhibition was measured by the binding energy of the best ligand pose measured in kcal/mol.
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Figure 4: Ligands used for docking

Natural Origin
T-1: Silibinin; T-2: Linarin; T-3: Tangeretin; T-4: Epigallocatechin gallate; T-5: Naringin; T-6: Daidzein

Synthetic Origin
T-7: Palbociclib (PD 0332991, pyridopyrimidine-derived cyclin-dependent kinase (CDK) inhibitor); T-8: Flavopiridol; T-9: Arcyriaflavin A; T-10: CINK4 (chemical inhibitor of Cdk4, triaminopyrimidine derivative)

Table 1: List of ligands taken for docking

<table>
<thead>
<tr>
<th>Compound</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-1</td>
<td>O. Baktiar Karim et.al.</td>
</tr>
<tr>
<td>T-2</td>
<td>M. Sivashannugham et.al.</td>
</tr>
<tr>
<td>T-3</td>
<td>Jen-Kun Lin et.al.</td>
</tr>
<tr>
<td>T-4</td>
<td>J. J. Chen et.al.</td>
</tr>
<tr>
<td>T-5</td>
<td>D. I. Kim et.al.</td>
</tr>
<tr>
<td>T-6</td>
<td>E. J. Choi et.al.</td>
</tr>
<tr>
<td>T-7</td>
<td>W. David Fry et.al.</td>
</tr>
<tr>
<td>T-8</td>
<td>A. Pumfrey et al.</td>
</tr>
<tr>
<td>T-9</td>
<td>G. Zhu et al.</td>
</tr>
<tr>
<td>T-10</td>
<td>R. Soni et al.</td>
</tr>
</tbody>
</table>

Validation of ligands by QSAR studies
In the present study, QSAR studies were performed using FAF Drugs: ADME/Tox filtering server for the determination of the inhibitor’s molecular properties such as Log P (partition coefficient), TPSA (topological polar surface area), Molecular weight, hydrogen bond acceptors and donors. TPSA, captured as the Vander Waals surface area of all nitrogen and oxygen atoms and their attached hydrogen atoms, was considered as an indicator for number of HB donors and acceptors. Calculated molecular properties and docking scores of all the compounds were shown in Table 2.

Table 2: Docking score and molecular properties of the ligands

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Energy value (kcal/mol)</th>
<th>MW</th>
<th>HD (OH+NH)</th>
<th>HA (O+N)</th>
<th>Log P</th>
<th>TPSA</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-1</td>
<td>-125.311</td>
<td>484.44</td>
<td>5</td>
<td>10</td>
<td>-1.37</td>
<td>161.35</td>
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<tr>
<td>T-2</td>
<td>-132.929</td>
<td>594.56</td>
<td>8</td>
<td>14</td>
<td>-2.74</td>
<td>219.29</td>
</tr>
<tr>
<td>T-3</td>
<td>-104.307</td>
<td>375.39</td>
<td>1</td>
<td>7</td>
<td>1.04</td>
<td>77.68</td>
</tr>
<tr>
<td>T-4</td>
<td>-146.356</td>
<td>458.37</td>
<td>8</td>
<td>11</td>
<td>0.83</td>
<td>199.44</td>
</tr>
<tr>
<td>T-5</td>
<td>-137.611</td>
<td>580.53</td>
<td>8</td>
<td>14</td>
<td>-1.71</td>
<td>227.13</td>
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<tr>
<td>T-6</td>
<td>-90.599</td>
<td>256.25</td>
<td>3</td>
<td>4</td>
<td>0.56</td>
<td>71.99</td>
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<tr>
<td>T-7</td>
<td>-138.856</td>
<td>449.55</td>
<td>3</td>
<td>9</td>
<td>2.21</td>
<td>106.51</td>
</tr>
<tr>
<td>T-8</td>
<td>-132.446</td>
<td>404.86</td>
<td>4</td>
<td>6</td>
<td>1.21</td>
<td>95.46</td>
</tr>
<tr>
<td>T-9</td>
<td>-124.515</td>
<td>329.35</td>
<td>5</td>
<td>5</td>
<td>2.69</td>
<td>84.07</td>
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<tr>
<td>T-10</td>
<td>-145.804</td>
<td>456.58</td>
<td>4</td>
<td>7</td>
<td>5.40</td>
<td>87.03</td>
</tr>
<tr>
<td>T-11</td>
<td>-145.556</td>
<td>447.53</td>
<td>3</td>
<td>7</td>
<td>4.99</td>
<td>80.85</td>
</tr>
</tbody>
</table>
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Figure 2: Crucial Interaction between compound T-10 (Red) and CDK4 protein (blue)

T-10, when analyzed showed a docking energy of -145.804 kcal/mol and very low TPSA value of 87.03. The crucial interaction of T-10 with CDK4 protein is shown in Figure 2. We have also observed that though compound T-4 has high dock score, due to high TPSA value and low Log P values, it cannot be considered as suitable CDK4 inhibitor. We have thus designed a new chemical compound, T-11, based on structure of T-4, which found to possess high dock score and low TPSA value compared to T-4. The structure of T-11 and crucial interaction of T-11 with CDK4 protein is shown in Figure 3 and 4. Thus with the least binding energy, least TPSA and Log P at all ensures T-10 and T-11 ligands to be good lead compounds for development of better CDK4 inhibitors.

Figure 3: Structure of T-11

Figure 4: Crucial Interaction between compound T-11 (Red) and CDK4 protein (blue)

CONCLUSION
In this study, we tried to explore the binding mechanism by applying molecular docking and correlated its docking score with the activity of the compounds taken. The results of our present study indicated that T-10 and T-11 are good leads for development of novel compounds that can be used as better CDK4 inhibitors. The compound T-10 has already been validated in vitro as well as in vivo while the compound T-11 has to be further validated in wet lab studies for its proper function.

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