

Research Article



Docking, Simulation Studies of Desulphosinigrin – Cyclin Dependent Kinase 2, an Anticancer Drug Target

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ABSTRACT

Cancer is a disease which causes death. Cyclin dependent kinases occupy a major role in the diagnosis and treatment of cancer. Desulphosinigrin a phytochemical from *Soymida febrifuga* seeds identified through GC-MS was used as a ligand and its inhibitory activity towards cyclin dependent kinase was observed by means of docking and simulation studies. Docking studies revealed the IC₅₀ value of Desulphosinigrin was 15.91 micro Molar and its binding energy was -6.55 Kcal/mol. Both the docked protein ligand complexes were refined and stabilized by molecular dynamic simulation studies in Desmond's. The simulation results, potential energy, and RMSD graph showed the protein ligand complexes are stabilization for 10n sec.

Keywords: Cyclin dependent kinase, Docking, Desulphosinigrin, phytochemical, Simulation.

INTRODUCTION

All eukaryotic cell basically depend on the cell cycle for its development and function. Cyclin dependent kinases (CDK) regulate the proliferation of eukaryotic cell through its serine, threonine kinases. CDK-2 is a regulator active during G1 phase and G1 TO S transition. Any abnormalities in regulator is associated with cancer. All kinases contain ATP binding pocket a site which is usually a target site by inhibitors (drug) through competitive inhibition. Drug discovery is a time, resource consuming process, requires complete screening. Hence, in order to minimize the time and effort as well as to have an idea or background knowledge about the drug of interest, the use of computational knowledge is a must and give useful data on prediction. One such important study is docking stimulation for binding of substrate with its receptor in order to predict the ligand and protein. The docking studies can be combined with molecular dynamics simulation studies for predicting good protein-ligand binding. Hence, the present study was planned to use Desulphosinigrin as a novel anticancer drug and its binding ability with cyclin dependent kinase was studied using simulation and docking.

MATERIALS AND METHODS

Preparation of Protein Target Structure

For the present study, the X-ray crystal structure of CDK2 in complex with ligand (PDB ID: 1AQ1) was obtained from Protein Data Bank. The protein preparation wizard facility has two components namely, preparation and refinement. After ensuring chemical accuracy, the preparation component adds hydrogen and neutralizes side chain that is neither close to binding cavity nor involve in formation of salt bridges. OPLS-AA force field was used for this purpose and then active site of protein was defined.

Ligand Selection

The ligand selected was Desulphosinigrin.¹ This was observed in highest level in the seeds of *Soymida febrifuga* when assessed for their phytochemicals through GC-MS. Hence, an attempt was taken to find its anticancer effect through docking and simulation studies, as it is time and cost saving process. The structure of compound is shown in Fig. 1.

Structure of Desulphosinigrin

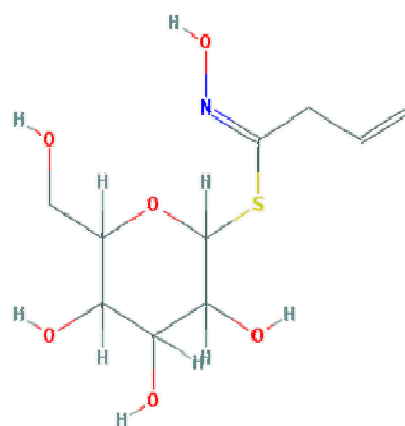


Figure 1: Docking, Simulation Parameters

Autodock 4.2^{2,3,4} was used as molecular docking tool in order to carry out simulations. Autodock helps to stimulate interactions between substrate or drug candidates as ligands and their macromolecular receptors of known three dimensional structures allowing ligand flexibility described to a full extent elsewhere. Flexibility of the ligand helps in exploring the spatial degrees of freedom for rotation and translation, for given number of torsional degrees of freedom. In the present study semi flexible docking was applied i.e. only one protein involved is kept flexible. The smaller protein especially ligand is



flexible and also smaller proteins will have more conformational variations as well as simulating the conformational changes of small protein is affordable.

MD simulations were carried out using Desmond v3.6^{5,6} and the images are rendered using Schrodinger's maestro v 9.5 vizualizer interface.⁷ Desmond is a high performance molecular dynamics simulations available for biomolecular systems using novel parallel algorithms and numerical techniques to achieve accuracy. Obtained scores, helps in analyzing the best binding pose. Individual particle motions and its interaction with other particles are measured through simulation studies. The stability of the protein was determined using root mean square deviation. Root mean square fluctuation measures the thermal motions of an individual residue and its fluctuations and it is defined as standard deviation of RMSD values. Radius of gyration indicates the compactness of the molecule during folding and unfolding. RMSD, Radius of gyration are the two indicators that indicate large structural changes in the protein. Rotable bond analysis analyses all single bonds in the structure. Bonds to hydrogen or simple substituents are excluded in the analysis as rotations of these bonds do not generate significantly different generations. In simple it identifies stable, non sense conformations.

RESULTS AND DISCUSSION

Molecular Docking Analysis

Molecular dynamics simulation results and graphs for the Desulphosinigrin-Cyclin Dependent Kinase 2 (PDB ID: 1AQ1) complex are discussed and shown below. The selected ligand was assessed for its binding affinity with the targeted protein. The results of the Docking studies are studied by the analysis of the number of hydrogen, hydrophobic interactions the ligand is formed with the receptor active site residues. The interaction strengths are scored. The ligand or compound giving the highest negative score is chosen as a best ligand and further analysis was performed by taking it with the receptor protein. The results with drug compound Desulphosinigrin was discussed below:

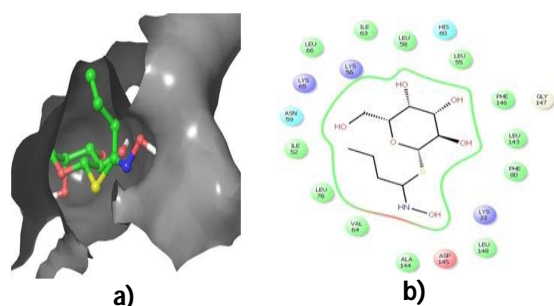


Figure 1: Illustration of 2D, 3D interactions of Desulphosinigrin targeting cyclin dependent kinase 2 for its anti cancer activity (Green – Van der waals interaction, Blue-Water, Violet-Covalent bond, Red-).

Docking snapshot of Desulphosinigrin compound for its anti-cancer activity targeting cyclin dependent kinase 2 is shown in Fig.1a and 1b. Fig.1a depicts the 3D surface area of the binding site of this ligand whereas, Fig.1b represents the 2D interactions of the compounds.

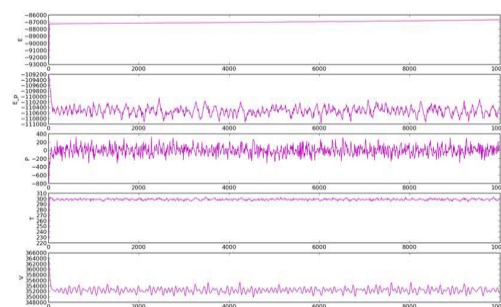


Figure 2: Simulation quality analysis. (E= Total Energy; EP = potential energy; P = pressure; T = temperature V = volume of the simulation box).

Simulation quality analysis is shown in Fig.2. Various panels in Fig.2 depicts that overall pressure (1 atm.), temperature (300 K) and volume (354000 \AA^3) of the MD simulated box is quite stable and maintained at the given conditions resulting for an averaged total energy of -87000 Kcal/mol and an averaged potential energy of -110400 Kcal/mol for the simulated Desulphosinigrin-Cyclin Dependent Kinase 2 complex.

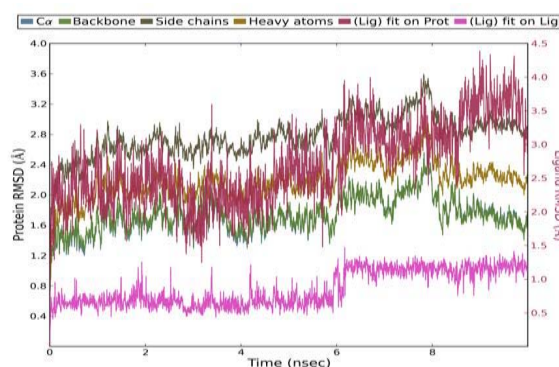


Figure 3: RMSD Analysis

The Molecular Dynamic analysis concerned with the stability of simulated cyclin dependant kinase protein-ligand during the simulation by monitoring the RMSD profiles as computed by the atomic displacements from Molecular Dynamic trajectories. Various lines in the Fig.3 depicts the RMSD parameters of protein as well as ligand with reference to cyclin dependent kinase 2 and Desulphosinigrin. Protein stability can be usually analyzed by its backbone RMSD (Green). From the Fig. 3 it is evident that the RMSD of the protein backbone in presence of the ligand has maintained around 2.0 angstrom units throughout the simulated time of 10 nanoseconds (10,000 picoseconds). On the other hand, RMSD of the ligand can also be seen from the Fig.3 Ligand (Desulphosinigrin) RMSD with reference to protein (Cyclin dependent kinase 2) was shown to be varying up to 3.2 angstroms whereas, ligand RMSD with reference to itself

shows quite stable at 0.4 angstroms up to 6 nanoseconds and later found at around 1.2 angstroms.

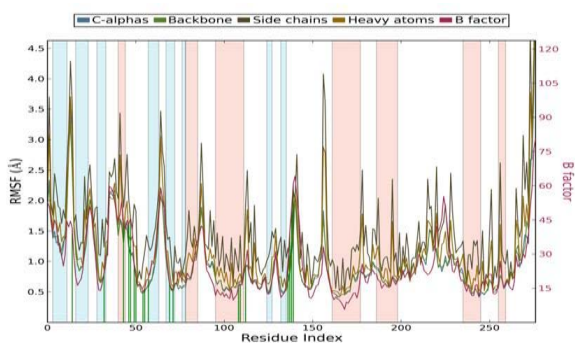


Figure 4: RMSF Analysis

Fig.4 shows the RMSF of the protein residues and ligand contacts (green lines).

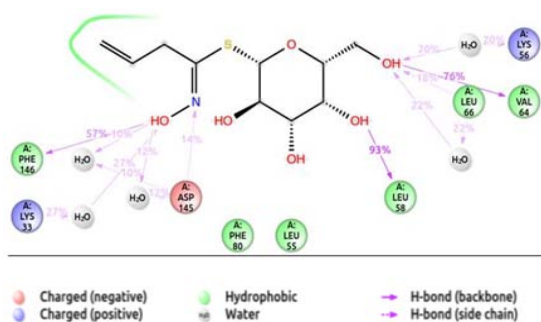


Figure 5: MD Simulation Snap

Fig.5 depicts various interacts formed with water molecules along with residues present in the protein with the percentage occupancy during the simulated time of 10ns.

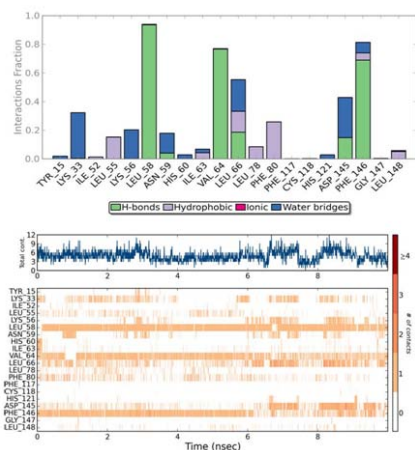


Figure 6: Inter Molecular Contacts

Fig.6 depicts the total number of inter molecular contacts (which includes hydrogen bonds; hydrophobic; ionic and water bridges) formed in between protein and ligand along the residues information involved in this contacts during the simulated time of 10ns.

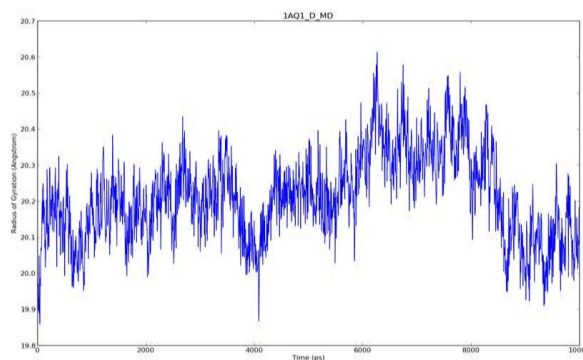


Figure 7: ROG Analysis

Fig.7 represents the radius of gyration (ROG) of the protein in presence of the ligand. ROG is considered as a parameter for the compactness of the given protein. From the above graphs it can be interpreted that the given protein in presence of the ligand was found to be fluctuating with reference to the simulation progress.

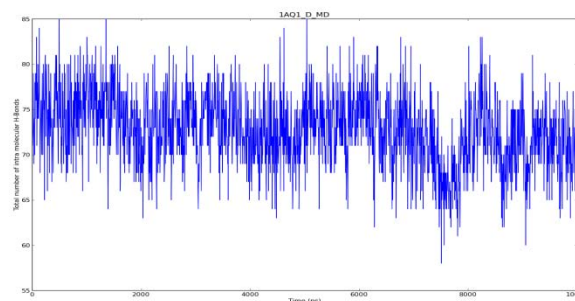


Figure 8: Intra molecular Hydrogen Bonds

Fig.8 shows the total number of intra molecular hydrogen bonds present in the protein during the simulation. Total number of intra molecular hydrogen bonds represents the rigidity/flexibility of the protein. From the above graphs it is evident that the total numbers of intra molecular hydrogen bonds were found to be maintaining an average of 75 in a range of 60 to 85.

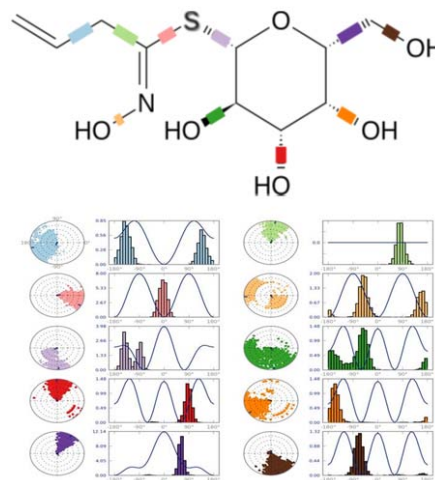


Figure 9: Rotatable Bond Analysis

Fig.9 demonstrates the rotatable bond analysis. Above graph shows the number of rotatable bonds present in the ligand and the degree of its freedom along with the

energy consumed for this rotation. 10 rotatable bonds were present in the present ligand (Desulphosinigrin) with various degrees of torsional freedom for each compound. The ligand torsions plot summarizes the conformational evolution of every rotatable bond (RB) in the ligand throughout the simulation trajectory (0.00 through 10.00nsec). The top panel shows the 2D schematic of a ligand with color-coded rotatable bonds. Each rotatable bond torsion is accompanied by a dial plot and bar plots of the same color. Dial (or radial) plots describe the conformation of the torsion throughout the course of the simulation. The beginning of the simulation is in the center of the radial plot and the time evolution is plotted radially outwards. The bar plots summarize the data on the dial plots, by showing the probability density of the torsion. If torsional potential information is available, the plot also shows the potential of the rotatable bond (by summing the potential of the related torsions). The values of the potential are on the left Y-axis of the chart, and are expressed in *kcal/mol*. Looking at the histogram and torsion potential relationships may give insights into the conformational strain the ligand undergoes to maintain a protein-bound conformation.

CONCLUSION

From the simulation studies, it is concluded that the compound Desulphosinigrin was found to have inhibitory activity on cyclin dependent kinase 2. This strongly contribute for its anticancer activity. Molecular interactions between the ligand Desulphosinigrin and cyclin dependant complex also seem to be highly stable as it has more hydrogen and more hydrophobic interactions. The molecular dynamics simulation studies too showed that the protein was steady throughout the total time scale of 3ns and the potential energy was also decreased. This signifies the fixed complex at constant temperature and pressure confirming the stable nature of protein-ligand complex. The results of docking analysis

we can conclude, that Desulphosinigrin might be used as a best possible therapeutic drug towards cancer treatment.

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