Original Article

Docking studies for screening anticancer compounds of Azadirachta indica using Saccharomyces cerevisiae as model system

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Abstract

Introduction:Plants have a long history of use in the treatment of cancer. Plant-derived compounds have played an important role in the development of several clinically useful anticancer agents. In the recent years, more emphasis has been placed on identifying plant-derived compounds that can be used as an effective treatment for life-threatening diseases such as cancer. Cancer is a disease where there is abnormal cell proliferation. The proliferation of the cancer cells are restrained by cdk/cyclin complexes, which control the normal process of cell cycle. **Materials and Method**: The current study involves the investigation of the anticancer property of the chemical compounds present in the leaves of Azadirachta indica by performing docking studies with the cell cycle control protein using Arguslab. **Result**: The compounds were docked with the cdk1 protein to identify suitable inhibitors against the protein function. **Conclusion**: The study were conducted on yeast Cdk protein, because these proteins showed homology with the human Cdks

Key words: ArgusLab, Azadirachta indica, cancer, cyclin-dependent kinase, docking

INTRODUCTION

Neem is one of the important native trees of India. It grows widely in most parts of India. There are nearly 16-20 million neem trees in India. Different parts of the neem tree are used in traditional medicine. Leaf decoction is effective against septic wounds, boils, and ulcers. Neem tree yields a variety of compounds belonging to chemical classes of diterpenes, limonoids, flavonoids, amino acids, and carbohydrates.[1-3] Approximately 300 compounds have been identified from the various parts of the neem tree. Neem compounds belong to diverse chemical classes, namely isoprenoids and non-Isoprinoids.[4-6]

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Cancer is the uncontrolled growth of abnormal cells in the body.[7,8] All cancers occur due to abnormalities in the DNA sequence. Mutations influence DNA sequence cell to alter from its normal type to a cancerous type. These somatic mutations alter the function of a critical gene, providing growth advantage to the cell, resulting in the emergence of an expanded clone. The identification of genes that are mutated has been a central aim of cancer research since the advent of recombinant DNA technology.[9] Cancer biology and its related studies have now led the way to find a solution to take control of the mutated genes.[9] The Cancer Genome Project has identified human gene sequence variants and mutations that play a critical role in the development of human cancers.[3] The evolution in the field of Cheminformatics has ushered in various approaches to identify the therapeutic properties of chemical compounds stored in the chemical databases.

Cyclin-dependent kinases (cdks) are a family of serine/threonine protein kinases whose members are small proteins (~34-40 kDa). Most of the known cyclin–cdk

complexes regulate the progression through the cell cycle. Animal cells contain at least nine cdkss; four of which, Cdk1, 2, 3, and 4, are directly involved in cell cycle regulation.[10-12] The role of the Cdks is to control cell cycle progression through phosphorylation of proteins that function at specific cell cycle stages. Cell cycle defects are often mediated by alterations in cdk activity. The treatment strategy lies in blocking the activity of cdk proteins that over-regulate the cell-cycle mechanism.

In the budding yeast *Saccharomyces cerevisiae*, the cell-cycle events are controlled by a single essential CDK called Cdk1/cdc28. The cdk function has been remarkably well conserved during evolution. It is possible, for example, for yeast cells to proliferate normally when their gene for cdk1 is replaced with the human one. This evidence clearly illustrates that cdk function in the cell-cycle control system has remained fundamentally unchanged over hundreds of millions of years of eukaryotic evolution.[10-12]

MATERIALS AND METHODS

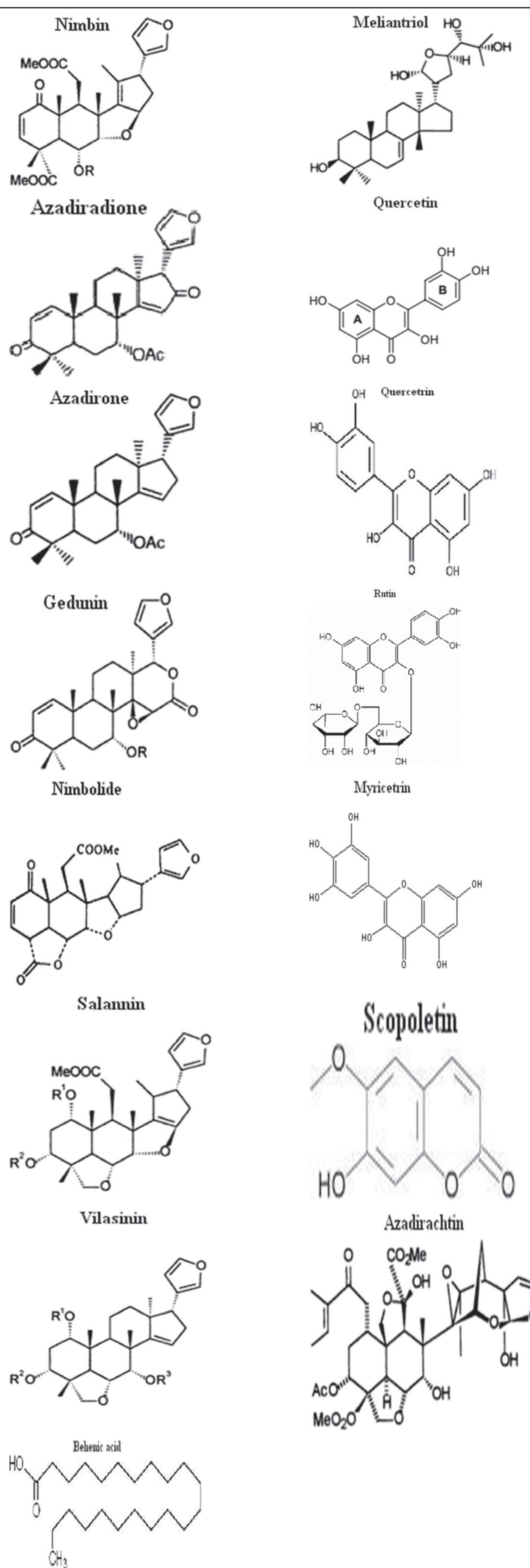
Preparation of protein structure

The 3D structure of cdk1_yeast protein (strain ATCC 204508/S288c), 298-amino-acid length sequence was used for the current study. The protein showed high similarity with the human cdk proteins and controlled all the stages of cell cycle. *Saccharomyces cerevisiae* is one of the best studied and a fascinating model organism to compare human cell machinery. The protein sequence was retrieved in the fasta format and the 3D structure was determined using CPH model server. All water molecules were removed and hydrogen atoms were added to the target protein molecule.

Preparation of the ligand structures

The ligands used for docking study were selected from the literature. The bioactive compounds that are mainly present in the leaves of *Azadirachtica indica* were considered for the study. The ligand structures were generated using the tool CORINA.[13] Three-dimensional optimizations of the ligand structures were done and saved as '.mol file'. Geometry optimizations of the ligands were performed according to the Hartree–Fock (HF) calculation method using ArgusLab 4.0.1 (Mark A. Thompson, Planaria Software LLC, Seattle, WA, USA, http://www.arguslab.com) software. The compounds included in the study are nimbin, azadiradione, azadirone, nimbolide, gedunin, salanin, vilasinin, behenic acid, meliantriol, quercetin, quercetrin, rutin, myricetrin, scopoletin, and azadirachtin. The bioactive compounds considered for the study are listed in Table 1.

Table 1: Compounds of Azadirachta indica and their molecular structures



Binding site predictionN

Q-SiteFinder (http://www.bioinformatics.leeds.ac.uk/qsitefinder) is used for binding site prediction.[14] It uses the interaction energy between the protein and a simple van der Waals probe to locate energetically favorable binding sites.

Protein-ligand docking using ArgusLab 4.0.1

ArgusLab is an electronic structure program that is based on the quantum mechanics. It predicts the potential energies, molecular structures; geometry optimization of structure, vibration frequencies of coordinates of atoms, bond length, and bond angle.

Cdk1_yeast protein was docked against the bioactive compounds from the leaves of neem tree using ArgusLab 4.0.1 (Mark A. Thompson, Planaria Software LLC, Seattle, WA, USA, http://www.arguslab.com).[15] The interaction was carried out to find the favorable binding geometries of the ligand with the protein. Docking of the protein ligand complex was mainly targeted only to the predicted active site. Docking simulations were performed by selecting "ArgusDock" as the docking engine. The selected residues of the receptor were defined to be a part of the binding site. A spacing of 0.4 Å between the grid points was used and an exhaustive search was performed by enabling "High precision" option in Docking precision menu, "Dock" was chosen as the calculation type, "flexible" for the ligand, and the "AScore" was used as the scoring function. A maximum of 150 poses were allowed to be analyzed; binding site box size was set to 20 × 20 × 20 Å so as to encompass the entire active site. The AScore function, with the parameters read from the AScore.prm file, was used to calculate the binding energies of the resulting docked structures. All the compounds in the dataset were docked into the active site of cdk1_yeast protein, using the same protocol. 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.

RESULTS AND DISCUSSION

Binding site of the protein

The ligand binding sites predicted by the Q-site finder server included the pockets regions comprising the amino acids, namely VAL 14, TYR 19, VAL 22, ALA 38, LYS 40, GLU 58, LEU 62, VAL 71, PHE 88, GLU 89, PHE 90, LEU 91, ASP 92, LEU 93, ASP 94, ARG 97, ASN 141, LEU 143, GLY 153, ASP 154, and PHE 155. The binding site selected comprised the site volume of 343 Å, where the total protein volume was 29022 Å.

Protein ligand interaction using ArgusLab 4.0.1

The ligand was docked with the target protein, and the best docking poses were identified. Figures 1-3 shows the binding poses of the compounds quercitin, quercitrin and salanin.

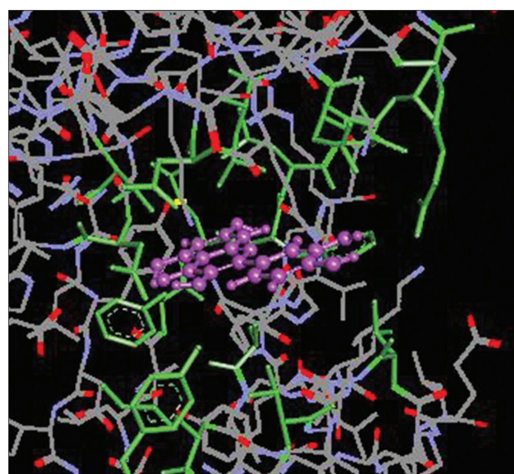


Figure 1: Binding pose of quercetin (pink) in binding site (green)

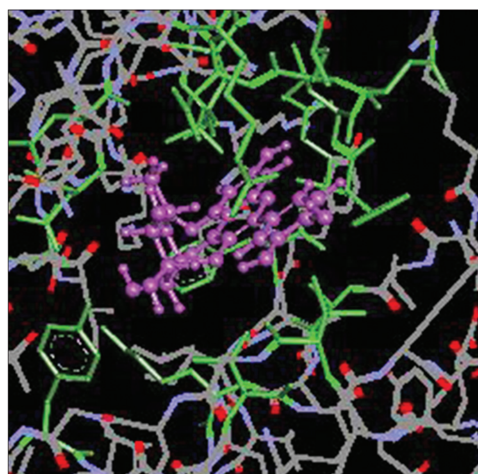


Figure 2: Binding pose of quercetrin (pink) in binding site (green)

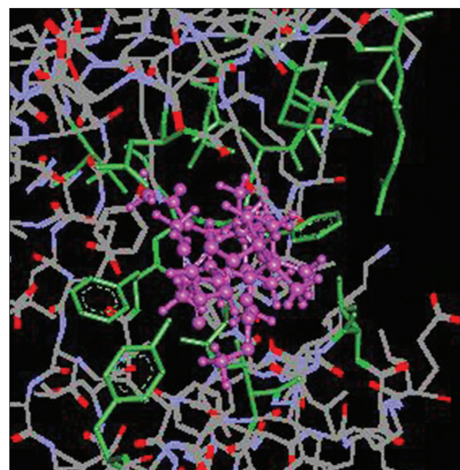


Figure 3: Binding pose of salanin (pink) in binding site (green)

Table 2: Docking score and molecular properties of bioactive compounds

Name of the compound	Best docking pose energy (Kcal/mol)
Nimbin	No binding pose
Azadiradione	−9.79189
Azadirone	−11.2438
Nimbolide	No binding pose
Gedunin	−10.4139
Salanin	−10.3339
Vilasinin	−10.3836
Behenic acid	−12.1412
Meliantriol	No binding pose
Quercetin	−8.91875
Quercetrin	−9.28441
Rutin	No binding pose
Myricetrin	−9.02589
Scopoletin	−8.87815
Azadirachtin	No binding pose

This best docking poses shows how the ligand molecule fits into the binding region of the target protein. Intermolecular flexible docking simulations were performed and energy values were calculated from the docked conformations of the cdk1_yeast protein–inhibitor complexes. Majority of the ligands had a greater binding affinity with the target protein cdk1_yeast protein. Inhibition was measured by the binding energy of the best ligand pose measured in kcal/mol. The binding pose and their energy are listed in Table 2.

CONCLUSION

All the compounds selected for the study are considered as orally safe compounds. A few compounds showed interaction with the cdc28 protein. A few compounds were not able to interact with the target protein. Thus the bioactive compounds that are interacting with the target can be used as a potent inhibitor to block the action of cdk protein. Thus the selected compounds can be verified at the clinical-level drug examinations and made into an effective anticancer drug.

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