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DOCKING STUDIES OF BCL-2 WITH *HEMIDESMUS INDICUS* COMPOUNDS FOR ANTI-CANCER STUDIES

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ABSTRACT

Medicinal plants have wide spread properties due to the presence of phytocompounds and are the alternative medicines available for thosewho cannot be helped by conventional medicine. In this work we have selected bioactive compounds from *Hemidesmus indicus* medicinal plant extracts. Gas chromatography and Mass Spectrum studies were studied to identify the compounds present in the ethanolic extracts based on the retention time and area. The identified compounds were used for anti-cancer activity by insilico method with BCL-2 which plays prominent role in causing cancer. Out of twenty selected compounds, docking results showed Methyl-1-Cyclo Hexane carboxylate and 1,2-diacetoxy-5-idohexane as best docked to the BCL-2.

KEYWORDS: Anticancer, BCL-2, Docking studies, *Hemidesmus indicus*, Insilico studies.

INTRODUCTION

Cancer is a global health problem with high morbidity and mortality and poses both economic and psychological challenges. Cancer cure and prevention therefore remain a high priority for the scientific community across the world. Insight gained into the etiology of cancer through various epidemiological studies encompassing various parameters such as geographical location, ethnicity, sex, age and trans-migratory populations have collectively revealed that lifestyle is one of the major influencing factors,[1-2] Other factors include environmental aspects such as automobile exhaust pollutants, solar UV radiation, occupational exposure to carcinogens[5] and mutagens, bacterial/viral infection, and genetic susceptibility[4,5] Lifestyle factors are usually classified as modifiable risk factors and include diet intake, smoking, caspase-3 activity[4] alcohol consumption, and physical activity and body mass. In general, physical activity instead of inactivity, abstinence from smoking and alcohol consumption, low body mass, and diets low in fat/calories are usually recommended for overall good health and have a positive influence on reducing the risk of cancer, especially breast and colorectal cancers.[2,6] Because all these factors can be modified, they also provide us with leverage to use them as interventive/preventive measures. Accordingly, the American Cancer Society has suggested guidelines on nutrition and physical activity for the prevention of cancerand early detection/screening for cancers of certain sites.[7]

From the epidemiological data indicating that dietary habits influence cancer risk, considerable scientific interest has been generated in developing various preventive measures based on diet,[1] especially those involving fruits and vegetables.[8-10] Fruits and vegetables, along with probiotics[3] belonging to plant kingdom, represent a vast source of phytochemicals of varied chemical structure; many of them have already been studied extensively for their potential anticancer or chemopreventive efficacy.[10] As such, interventions based on fruits and vegetables are not only "more natural" in lowering cancer risk without posing "any side effects" but also in maintaining good general health based on the fact that they are major sources of vitamins, minerals, and fiber. The presumptive results may also lead to cloning of the compounds for detailed studies in yeast model systems like Pichiapastoris.[2] In this work, we have focused our discussion on recent advancements largely in Hemidesmus indicus root extract regarding their cancer chemopreventive and anticancer efficacy and associated molecular mechanisms.

METHODOLOGY

GC-MS method for identification of compounds

GC-MS analysis was carried out on a GC CLARUS 550 PerkinElmer system comprising a gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument employing the following conditions: Column Elite-1 fused silica capillary column (30×0.25 mm ID×1EM df, composed of 100% Dimethyl poly siloxane), operating in electron impact mode at 70 eV; helium (99.999%) was used as carrier gas at a constant flow of 1ml/min and an

injection volume of 0.5 EI was employed with split ratio of 10:1injector temperature 250°C; ion-source temperature 280°C. The oven temperature was fixed from 110°C (isothermal for 2 min), with an increase of 10°C/min, to 200°C, then 5°C/min to 280°C, ending with a 9 min isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 s and fragments from 40 to 550 Da.

BCL-2Active site Identification

The structure of BCL-2 (PDB: 1GJH) was retrieved from PDB database and unnecessary chains, hetero atoms were removed using SPDBV software, hydrogens were added to the protein and used for active site identification.

Active site of BCL-2 of Homo sapiens was identified using CAST p server. A new program, CAST p, for automatically locating and measuring protein pockets and cavities, is based on precise computational geometry methods, including alpha shape and discrete flow theory. CAST p identifies and measures pockets and pocket mouth openings, as well as cavities. The program specifies the atoms lining pockets, pocket openings and buried cavities; the volume and area of pockets and cavities; and the area and circumference of mouth openings.

Docking method

Docking was carried out using GOLD (Genetic Optimization of Ligand Docking) software which is based on genetic algorithm which allows as partial flexibility of protein and full flexibility of ligand. The compounds identified in GC-MS are docked to the active site of the BCL-2 of Homo sapiens. The interaction of the compounds with the active site residues are thoroughly studied using molecular mechanics calculations. The parameters used for GA were population size (100), selection pressure (1.1), number of operations (10,000), number of island (1) and niche size (2). Operator parameters for crossover, mutation and migration were set to 100, 100 and 10 respectively. Default cutoff values of 3.0 A° (dH-X) for hydrogen bonds and 6.0 A° for vanderwaals were employed. During docking, the default algorithm speed was selected and the ligand binding site in the targets were defined within a 10 A° radius with the centroid as CE atom of active residues. The number of poses for each inhibitor was set 100, and early termination was allowed if the top three bound conformations of a ligand were within 1.5A° RMSD. After docking, the individual binding poses of compounds were observed and their interactions with the protein were studied. The best and most energetically favorable conformation of ligands was selected.

Gold Score fitness function

Gold Score performs a force field based scoring function and is made up of four components: 1. Protein-ligand hydrogen bond energy (external H-bond); 2. Protein-ligand vander Waals energy (external vdw); 3.Ligand internal vander Waals energy (internal vdw); 4.Ligand intramolecular hydrogen bond energy (internal- H-bond). The external vdw score is multiplied by a factor of 1.375 when the total fitness score is computed. This is an empirical correction to encourage protein-ligand hydrophobic contact. The fitness function has been optimized for the prediction of ligand binding positions.

GoldScore = S (hb_ext) + S (vdw_ext) + S (hb_int) + S (vdw_int)

Where S (hb_ext) is the protein-ligand hydrogen bond score, S (vdw_ext) is the protein-ligand van der Waals score, S (hb_int) is the score from intramolecular hydrogen bond in the ligand and S (vdw_int) is the score from intramolecular strain in the ligand.

RESULTS AND DISCUSSION

From the PDB databank, the PDB files were collected and the final stable structure of the BCL-2 of Homo sapiens obtained is shown in Figure 1. The ligands present in the crystal structure were removed along with hetero atoms for docking studies.

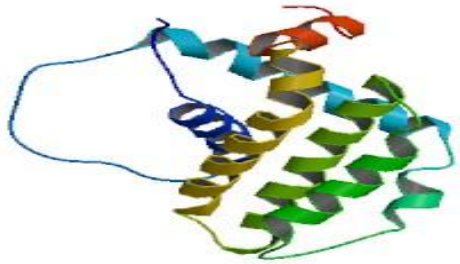

Fig. 1: Structure of BCL-2 retrieved from Protein data bank with seven helices.

Active site Identification

After the final model was built, the possible binding sites of BCL-2 was searched based on the structural comparison of template and the model build and also with CASTP server and was shown in Figure 2. Infact from the final refined model of BCL-2 domain using SPDBV program, it was found that secondary structures are highly conserved and the residues shown below.

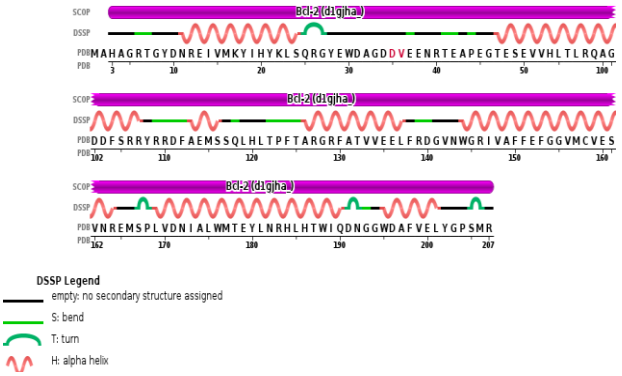

Fig. 2: Amino acids in the active site region (red colour) of the BCL-2protein.

Docking of inhibitors with the active site

Docking of the compounds with BCL-2 was performed using GOLD 3.0.1, which is based on genetic algorithm. This program generates an ensemble of different rigid body orientations (poses) for each compound conformer within the binding pocket and then passes each molecule against a negative image of the binding site. Poses clashing with this 'bump map' are eliminated. Poses surviving the bump test are then scored and ranked with a Gaussian shape function. We defined the binding pocket using the ligand-free protein structure and a box enclosing the binding site. This box was defined by extending the size of a cocrystalized ligand by 4A. This dimension was considered here appropriate to allow, for instance, compounds larger than the cocrystallized ones to fit into the binding site. One unique pose for each of the best-scored compounds was saved for the subsequent steps. The compounds used for docking was converted in 3D with SILVER. To this set, the substrate corresponding to the protein was added. Docking of best inhibitor with the active site of protein showed the activity of the molecule on protein function.

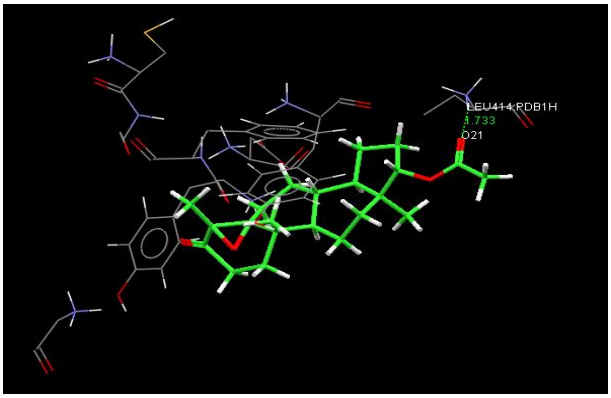

Fig. 3: Methyl-1-Cyclo Hexane carboxylate docked to BCL-2 active site

In docking studies, Methyl-1-Cyclo Hexane carboxylate showed a docking energy of 22.56K.cal/mol with BCL-2. In docking, Methyl-1-Cyclo Hexane carboxylate (O21) docked to LEU414 with a bond length of 1.733A^{o}.

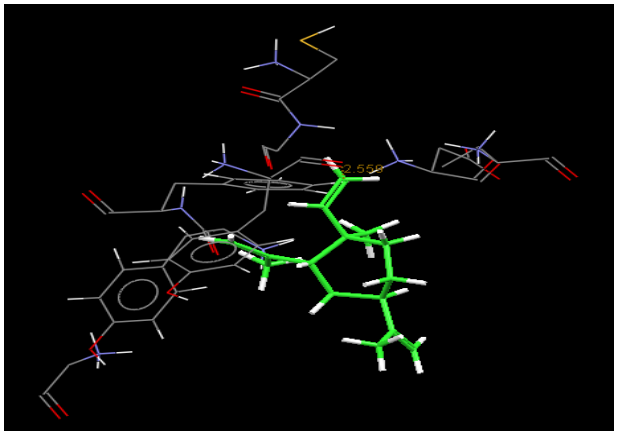

Fig 4: 1, 2-diacetoxy-5-idohexanedocked to BCL-2 active site.

In docking studies, 1,2-diacetoxy-5-idohexane showed a docking energy of 20.24K.cal/mol with BCL-2. In docking, 1,2-diacetoxy-5-idohexane (H33) docked to LEU414 with a bond length of 2.559A^{o}.

CONCLUSION

From the studies, we concluded that GC-MS analysis identified twenty phyto compounds from *Hemidesmus indicus* root ethanolic extract. The identified phytocompounds were checked for their anti-cancer activity using insilico method. BCL-2 protein was retrieved from the database and its active site was identified using CASTp server. The twenty phytocompounds were docked to the BCL-2 for their anti-cancer activity. Among the phytocompounds docked, Methyl-1-Cyclo Hexane carboxylate showed a docking energy of 22.56K.cal/mol and 1,2-diacetoxy-5-idohexane showed 20.24K.cal/mol with BCL-2.From these docking studies we can conclude that among the phytocompounds identified, these two compounds have good BCL-2 inhibitory activity.

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