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In silico Study on Anticancerous Impact of Compounds Isolated from *Sargassum wightii* by Blocking Bcl-xL

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Abstract

Cancer is one of the major life threatening diseases of today's era. Algae can be used as one of important medical source against this disease due to its antioxidant, anticancer and antiviral properties. *Sargassum wightii* is one of the important species of brown algae with wide range of bioactive properties. This genus is an ideal target for investigating the presence of bio-molecules for various medical and industrial applications. Molecular docking is a key tool in structural molecular biology. Docking can be used to perform virtual screening of compounds, rank the results, and to propose structural hypotheses of how the ligands inhibit the target. In the present study, docking is implemented to find the affinity of compounds with Bcl-xL through Hex. Among the four compounds studied, Phytol has least E-value -315.39 which binds to the BH3 domain of Bcl-xL protein. Diethyl phthalate have E-value -200.91 that bound to the BH4 domain of Bcl-xL. Methyl salicylate and Benzoic acid neither bind with BH3 nor to BH4. Hence Phytol and Diethyl phthalate possessed highly effective and stable complex with Bcl-xL protein and it can induce apoptosis in cancerous cells. Further studies are needed to elucidate its mechanism in *in vitro* conditions.

Keywords: Anti-cancerous, docking, Hex, Bcl-xL, Phytol, Diethyl phthalate, Methyl salicylate, Benzoic acid, E-value.

Introduction

Algae are the primary producers of organic matter in the aquatic environment because of their photosynthetic activity. Algae can also be used as one of the important medical sources due to its antioxidant, anticancer and antiviral properties. The therapeutic properties of algae are used for the promotion of health. Algae in both micro and macro forms have tremendous scope for exploring bioactive compounds. They have been used traditionally for medicinal purpose in India, China, Japan, Korea, Ireland, Wales and other countries. The phaeophycean algae *Sargassum* contain novel fucoidines, which have anti-inflammatory, anti-tumoral, anti cancerous and immunostimulant activities (Itoh *et al*., 1993; Gabriele *et.al* 1994).

During the cancer multi-stage cascade, normal cells undergo initiation, promotion, and progression processes. Extensive researches on the cellular and molecular basis of the carcinogenesis cascade provide a targeted approach for cancer chemoprevention, which aims to reverse the development and progression of precancerous cells through the use of non cytotoxic doses of nutrients or pharmacological agents. Theisen (2001) extracted different fractions of watersoluble polysaccharide from *Sargassum wightii* which have cancer chemopreventive activity that prevent different stages of carcinogenesis process.The compounds which are derived from algae are recommended as potential drug targets to treat cancer and angiogenesis-related diseases. Seaweeds are the most important reservoirs of new therapeutic compounds for human beings. Various compounds extracted from seaweeds have eradicated or reduced the progression of cancer. The present study was aimed to find the binding affinity of the compounds isolated from *Sargassum wightii* such as Phytol, Diethyl phthalate, Methyl salicylate and Benzoic acid with the target Bcl-xL which is over-expressed in cancerous cells through computational docking by using Hex.

Materials and Methods

In the present study online tools like Pubchem and PDB as well as offline tools like Rasmol, Hex and Swiss PDB Viewer were used. Pubchem: The structure of compounds such as Phytol, Diethyl phthalate, Methyl salicylate and Benzoic acid isolated from *Sargassum wightii* were collected from structure database called Pubchem, which is publicaly availble. The mission of PubChem is to deliver free and easy access to all deposited data, and to provide intuitive data analysis tools (Sayers *et al*., 2011). Protein Data Bank (PDB) : The three dimensional structure of Bcl-xL protein was taken from Protein Data Bank (PDB).The 3D structure obtained through X-ray diffraction,NMR experiment and MS method were deposited in this database (Joel *et al*., 1998). Rasmol: RasMol is a free, interactive molecular-graphics viewer. The program reads in the 3-D coordinates for a molecule using the pdb file format. It displays the molecule in various representations and allows one to rotate the molecule interactively. In the present study, the molecules and protein were viewed through this efficient tool. Hex: Hex is an interactive molecular

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graphics program for calculating and displaying feasible docking modes for pairs of protein and compound. Hex can also calculate protein ligand docking, assuming the ligand is rigid, and it can superpose pairs of molecules using only knowledge of their 3D shapes. The parameters used in Hex for the docking process were; Correlation type – Shape only, FFT Mode – 3D fast lite, Grid Dimension – 0.75, Receptor range – 180, Ligand Range – 180, Twist Range 360, Distance Range 40. It uses Spherical Polar Fourier (SPF) correlations to accelerate the calculations and it is one of the few docking programs which have built in graphics to view the result (Alex *et al.,* 2005). Swiss-PdbViewer: It is an application that provides a user friendly interface which allows analyzing several proteins at the same time. The proteins can be superimposed in order to deduce structural alignments and compare their active sites or any other relevant parts. The functional site of the protein was identified through this software.

Results and Discussion

Bioinformatics is seen as an emerging field with the potential to significantly improve how drugs are found, brought to the clinical trials and eventually released to the marketplace. Rasmol is a powerful educational tool for showing the structure of DNA, proteins and smaller molecules. Secondary structures are easily identified within this format. Tony (2000) generated ribbon diagram of the 1AZF pdb file illustrating the a-helical and b-sheet regions of the HEW lysozyme molecule using Rasmol. In the present study, the Figure 1 represents the secondary structure of Bcl-xL. The ball and stick model of the compounds are shown in Figure 2 a,b,c, and d.

Docking is one of the powerful methods to find the binding affinity of the receptor with the compounds. Kazemi *et.al.,*(2013) studied the Human serum albumin receptor and the commercially available drug pain reliever. The receptor was docked to the drug and the energy value obtained were recorded as ibuprofen (-220.34) and aspirin (199.67) using the HEX docking software. Depending on the energy values, the best drug chosen was ibuprofen. Rupanjali *et al.,* (2013) conducted the quinine based analogs which were docked to the receptor plasmepsin II (1lee) using the docking software HEX. From the Docking study of 14 compounds, three compounds exhibited better affinity towards the receptor. In the present study, four compounds which are derived from *Sargassum wightii* were docked with receptor Bcl-xL and computed energy values using Hex. Energy value of Benzoic acid is -175.27, Diethyl phthalate -261.83, Methyl salicylate -200.91 and Phytol is -315.39. The energy values obtained were found to be vary between -175 to -315. Benzoic acid shows high energy value and hence exhibited less stablility, whereas Phytol possessed less energy value and therefore its stability is very high. On the basis of energy values Phytol is taken as best compound among the four compounds as it showed good binding affinity towards the receptor Bcl-xL.

Figure 1. The secondary structure of protein Bcl-xL

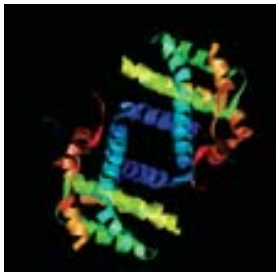


Figure 2.3D view of (a)Benzoic acid, (b)Methyl salicylate, (c)Diethyl phthalate and(d)Phytol

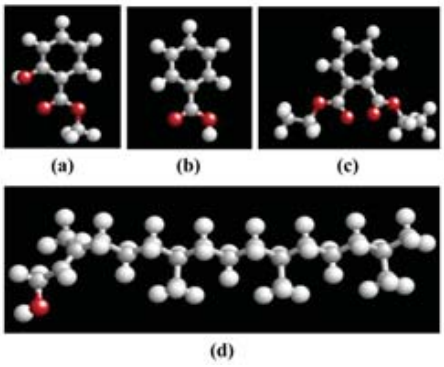
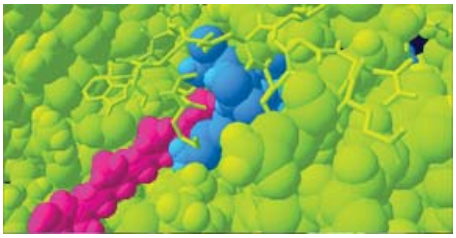


Figure 3. Phytol (pink) bound with the BH3 domain (blue) of Bcl-xL



Mukerjee *et al*., (2010) established that inhibition of anti-apoptotic members of the Bcl-2 family is an intervening target for the development of anti-cancer therapy. The active site of the target anti-apoptotic proteins possess three domains namely BH1, BH2, BH3. Interaction of the BH3 domain with Bcl-2 related death suppressors such as Bcl-xL could be critical for its death activity in cells. Further it was proved by *Mohamad et al*.,(2008), that a small molecule that interact with the BH3 binding domain of Bcl-xL/ Bcl-2 will function as Bcl-xL/ Bcl-2 antagonists and promote apoptosis. In connection to this context in our present study compound Phytol inhibiting Bcl-Xl receptor also serve to bind to BH3 binding groove of Bcl-xL receptor. The result goes in accordance with the work of Christopher *et al.,*(2005) and Logberg *et al*., (2007) who worked on *in silico* docking studies using the three dimensional structure of Bcl-xL predicted gossypol to bind into the deep hydrophobic groove on the surface of Bcl-xL receptor. The major mode of action of gossypol is through its interaction with the BH3 binding groove in Bcl-xL to a lesser extent and thereby preventing the interaction with the proapoptotic proteins and allow mitochondrial permeabilization which is essential for the release of cytochrome c and its interaction with APAF-1 by activating the caspase cascade and lead to apoptosis. In the present study, among the four compounds studied, Methyl salicylate, Diethyl phthalate and Benzoic acid did not dock in the BH3 domain of the receptor. But the Phytol made complex with the BH3 domain of the Bcl-xL and hence it could be able to induce apoptosis or facilitate apoptosis induction in cancer cells. In conclusion, Phytol is said to be a promising anti-cancerous compound.

Conclusion

The present study was carried out to establish the affinity of the compounds isolated from the algae *Sargassum wightii* with Bcl-xL through docking studies and binding site analysis. Among the four compounds from the algae, Phytol showed excellent binding caliber against Bcl-xL and found to be a good anti-cancer agent.

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