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In silico molecular docking of astaxanthin and sorafenib with different apoptotic proteins involved in hepatocellular carcinoma

V. Suganya, V. Anuradha*

PG and Research Department of Biochemistry, Mohamed Sathak College of Arts & Science, Sholinganallur, Chennai, Tamil Nadu, India

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ABSTRACT

Hepatocellular carcinoma (HCC) is the most common type of primary liver cancer. Several signaling pathways are involved in Hepatocellular carcinoma. Sorafenib is used as positive drug for Hepatocellular carcinoma. Astaxanthin is a keto carotenoid with pinkish red colour found in various sea foods, shrimp, salmon, crab and lobster which have major role as anticancer drug. I*n silico* molecular docking was performed to Astaxanthin and Sorafenib with growth factor receptors like VEGFR2, EGFR; apoptotic proteins like Bcl2, Caspase 3 and Caspase 9 by computational method to prove the mechanism of Astaxanthin and Sorafenib as anticancer agent. Our study, suggest that better docking score was obtained by astaxanthin when compared to sorafenib the positive drug. Hence, astaxanthin can be used as a potent drug against various HCC drug targets.

1. Introduction

Hepatocellular carcinoma (HCC) is the primary cancer of the liver, derived from hepatocytes and occurs approximately 80% of cases of liver cancer (Jemal et al., 2011). It is the fifth common cancer in the world, and the third most common cancer in adult male population (Ali Ben Mousa, 2008). Approximately 7.5 Lakhs of new cases of HCC per year occurs globally which makes HCC as the 5th common cause of cancers effecting human. The mortality in HCC is very high; about 7 Lakhs death due to HCC occur annually and has been estimated to be 3rd common cause of death due to cancers effecting human (EASL–EORTC Clinical, 2012) (see Table 1).

HCC is most commonly develops in people associated with liver disease, particularly in people with chronic hepatitis B and C. Symptoms often don't appear in the early stages but in later stage, symptoms include weight loss, upper abdominal pain or yellowing of the skin (Jaundice). Treatments include surgery, transplant, freezing or heating the cancer cells and chemotherapy (Balogh et al., 2016).

Signaling pathways have become a major source of targets in HCC. Survival benefits have been achieved with sorafenib, a multikinase inhibitor which is approved for the treatment of primary kidney cancer (advanced renal cell carcinoma), advanced primary liver cancer (hepatocellular carcinoma), and radioactive iodine resistant advanced thyroid carcinoma. Sorafenib is a synthetic compound targeting growth signaling and angiogenesis. Sorafenib blocks the enzyme RAF kinase, a critical component of the RAF/MEK/ERK signaling pathway that controls cell division and proliferation. Sorafenib in addition, it inhibits the VEGFR-2/PDGFR-beta signaling cascade (Fig. 1), thereby blocking tumor angiogenesis (Ali Ben Mousa, 2008; Wang and Sun, 2015).

Astaxanthin is a member of the xanthophylls, because it contains not only carbon and hydrogen but also oxygen atoms. Astaxanthin consists of two terminal rings joined by a polyene chain. This molecule has two asymmetric carbons located at the 3, 3′ positions of the β-ionone ring with hydroxyl group (−OH) on either end of the molecule. In case one, hydroxyl group reacts with a fatty acid then it forms monoester, whereas when both hydroxyl groups are reacted with fatty acids the result is termed a di-ester. Astaxanthin exists in stereoisomers, geometric isomers, free and esterified forms (Higuera-Ciapara et al., 2006). All of these forms are found in natural sources. The stereoisomers (3*S*, 3′*S*) and (3*R* 3′*R*) are the most abundant in nature. *Haematococcus* biosynthesizes the (3*S*, 3′*S*)-isomer whereas yeast *Xanthophyllomyces dendrorhous* produces (3*R*, 3′*R*)-isomer (Hussein et al., 2006). Synthetic astaxanthin comprises isomers of (3*S*, 3′*S*) (3*R*, 3′*S*) and (3*R*, 3′*R*). The primary stereoisomer of astaxanthin found in the Antarctic krill *Euphausia superba* is 3*R*, 3′*R* which contains mainly esterified form, whereas in wild Atlantic salmon it is 3*S*, 3′*S* which occurs as the free form (Foss et al., 1987). Astaxanthin is a red colour pigment belongs to the family xanthophyll's; the oxygenated derivatives of carotenoids. Mainly found in many microalgae like *Haematococcus pluvialis,* the red yeast *Phaffia rhodozyma* etc., it is also founded in various sea foods like shrimp, salmon, crab and lobster. Astaxanthin has potential health-promoting effects in the prevention and treatment of

* Corresponding author.
E-mail address: vanuradha2712@gmail.com (V. Anuradha).

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Table 1
Compound name with PubChem CID for molecular docking.

S.NO	Compound name	PubChem CID	Molecular formula	Molecular weight
1	Astaxanthin	5281224	$C_{40}H_{52}O_4$	596.852 g/mol
2	Sorafenib	216239	$C_{21}H_{16}ClF_3N_4O_3$	464.829 g/mol

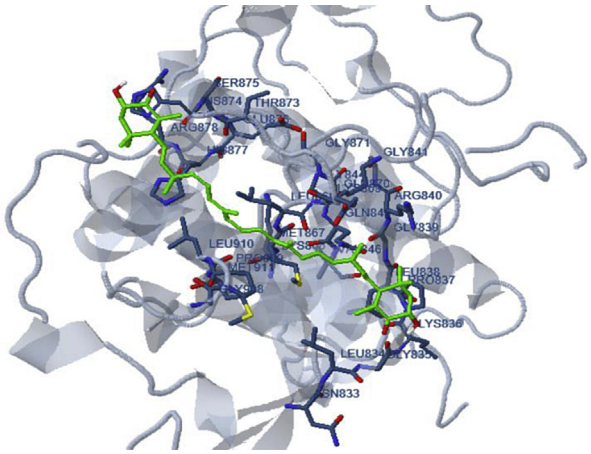

Fig. 1. Molecular docking of Astaxanthin and 2OH4.

various diseases, which includes cancers, diabetes, cardiovascular diseases, gastrointestinal diseases, liver diseases, neurodegenerative diseases, eye diseases, skin diseases, exercise-induced fatigue, male infertility, and renal failure (Dhankhar et al., 2012).

The anti-cancer effects of astaxanthin are reportedly attributed to its effects on the pathological process of cancer cells through a variety of pathways including apoptosis, inflammation and cell junction. Astaxanthin inhibits proliferation and induces apoptosis of hepatocellular carcinoma cells by signaling mechanism that activates the transcription of Bax and Bcl2 (Li et al., 2015).

Sorafenib (Nexavar) is a niacinamide and phenylurea derivative that inhibits multiple intracellular and cell surface kinases thought to be involved in angiogenesis, including RAF kinases and VEGF receptors. It is used in the treatment of advanced renal cell carcinoma and hepatocellular carcinoma, and for treatment of thyroid carcinoma refractory to radioactive iodine therapy. Sorafenib is a Kinase Inhibitor. The mechanism of action of sorafenib is as a Protein Kinase Inhibitor. Sorafenib is a synthetic compound targeting growth signaling and angiogenesis. Sorafenib blocks the enzyme RAF kinase, a critical component of the RAF/MEK/ERK signaling pathway that controls cell division and proliferation; in addition, sorafenib inhibits the VEGFR-2/PDGFR-beta signaling cascade, thereby blocking tumor angiogenesis.

In 2005, Phase I clinical studies were undertaken to establish the pharmacokinetics and safety of sorafenib (Strumberg et al., 2005). Flow cytometry showed that Erk phosphorylation was significantly decreased at doses above 200 mg ($P < 0.01$) when treated with sorafenib. The Phase II trials in 2006 measured the efficacy, toxicity, pharmacokinetics, and biomarkers of sorafenib in advanced HCC patients (clinicaltrials.gov, NCT00044512) (Abou-Alfa et al., 2006). The modest efficacy of sorafenib suggested its use in combination with other anticancer drugs. In 2008, Phase III trials were conducted by the Sorafenib Hepatocellular Carcinoma Assessment Randomized Protocol (SHARP) Investigators Study Group in a double-blind, placebo controlled manner (clinicaltrials.gov, NCT00105443) (Llovet et al., 2008). The study administered 400 mg of sorafenib or a placebo bid to 602 patients with advanced HCC and no prior systemic treatment. Sorafenib increased the median survival time by approximately 3 months (the median for sorafenib was 10.7 months, while the median for the placebo group was 7.9 months). The median time to symptomatic progression was approximately equal in both groups. However, the median time to radiologic progression was 5.5 months in the sorafenib group and only 2.8 months in the placebo group ($P < 0.001$). Only 2% of patients in the sorafenib group had a partial response, as defined by a 30% decrease in the sum of tumor diameters. The overwhelming majority of sorafenib patients (71%) had stable disease. In the placebo group, 1% of patients had a partial response and 67% had stable disease. The sorafenib group was also more likely to experience diarrhea, weight loss, hand-foot syndrome, and hypophosphatemia than those in the placebo group. The SHARP trials concluded that sorafenib was effective in increasing survival time, but the data shows that tumor response rates were very low. In the same year, Bruix et al., (2012) analyzed the data from the SHARP trial to determine if patients with macroscopic vascular invasion (MVI) or extrahepatic spread (EHS) responded differently to sorafenib compared to those without these complications. In order to confirm the results of the SHARP trials, a second phase III study was conducted in the Asia-Pacific Region (clinicaltrials.gov, NCT00492752) (Cheng et al., 2009). This region contains the most cases of HCC because it has a high prevalence of chronic hepatitis B infection. The trials randomly divided 271 patients with advanced HCC and no previous systematic therapy into two groups: sorafenib (226 patients) and placebo (76 patients). The patients were administered oral sorafenib or a placebo two times a day in six week cycles.

The median survival of patients was 6.5 months in those taking sorafenib but only 4.2 months for those with the placebo. In addition, the time to progression was twice as long in the sorafenib patients (2.8 months) as in the placebo patients (1.4 months). Thus, the Asia-Pacific trials confirmed the efficacy of sorafenib found in the SHARP trials. Since sorafenib has been proved in its efficacy, the present study compared the docking efficiency of astaxanthin with sorafenib as standard.

Thus, in the present study the growth factors like VEGFR2, EGFR; apoptotic proteins like bcl2, caspase 3 and caspase 9 were selected for docking with astaxanthin and standard drug sorafenib using computational methods to prove their anticancer activity in hepatocellular carcinoma.

2. Materials and methods

The 3D structure of astaxanthin and positive drug sorafenib was retrieved from PubChem (https://pubchem.ncbi.nlm.nih.gov/). Similarly the 3D structures of different apoptotic proteins were retrieved from Protein data bank (www.rcsb.org; Table 2). The molecular docking process was also carried out with sorafenib as positive drugs for HCC, which was compared with the binding efficiency of Astaxanthin drug.

Docking calculations were carried out using Docking Server (https://www.dockingserver.com/web) (Bikadi and Hazai, 2009). Gasteiger partial charges were added to the ligand atoms. Non-polar hydrogen atoms were merged, and rotatable bonds were defined. Docking calculations were carried out on ***Astaxanthin*** protein model. Essential hydrogen atoms, Kollman united atom type charges, and solvation parameters were added with the aid of AutoDock tools (Morris and Goodsell, 1998). Affinity (grid) maps of × × Å grid points and 0.375 Å spacing were generated using the Auto grid program

Table 2
Growth factors and apoptotic proteins with PDB ID.

S.NO	Target	PDB ID
1	VEGFR-2	2OH4
2	EGFR	4LQM
3	Bcl-2	2W3L
4	Caspase 3	1GFW
5	Caspase 9	2AR9

Table 3
Docking parameters.

S.NO	Protein Clean	
1	***tstep***	0.2
2	***qstep***	5.0
3	***dstep***	5.0
4	***rmstol***	2.0
5	***ga_pop_size***	150
6	***ga_num_evals***	250000
7	***ga_num_generations***	540000
8	***ga_run***	10

(Morris and Goodsell, 1998). Auto Dock parameter set- and distance-dependent dielectric functions were used in the calculation of the van der Waals and the electrostatic terms, respectively. Docking simulations were performed using the Lamarckian genetic algorithm (LGA) and the Solis & Wets local search method (Solis and Wets, 1981). Initial position, orientation, and torsions of the ligand molecules were set randomly. All rotatable torsions were released during docking. The overall binding potential was assessed by the interaction profile and binding energy of Astaxanthin when compared with standard anticancer drug (Sorafenib) (see Table 3).

Each docking experiment was derived from 10 different runs **(*ga_run*)** that were set to terminate after a maximum of 250000 energy evaluations **(*ga_num_evals*)**. The population size was set to 150 **(*ga_pop_size*)**. During the search, a translational step of 0.2 Å **(*tstep*)**, and quaternion and torsion steps of 5 **(*qstep*)** were applied.

3. Results and discussion

Molecular docking of test sample astaxanthin and the positive drug sorafenib was carried out by interaction with VEGFR, EGFR, Bcl-2, Caspase 3 and Caspase 9. The minimum binding energy confirms that apoptotic proteins were successfully docked with astaxanthin and sorafenib (Table 4). Figs. 1–10 represents the docking output of the test samples. Interaction between the apoptotic protein and the sample was given in Tables 5–14.

The amino acid residues leu834, pro837, met867, leu868, his874, his877, pro909 of the VEGFR-2 shows only hydrophobic interaction with astaxanthin molecule whereas the residues lys824, asp855, leu900 forms H-bond with sorafenib. The interaction between protein and sorafenib also includes polar, hydrophobic bonding by different residues like asp855, lue900 (Tables 5 and 6). Astaxanthin shows good binding affinity with bonding energy of −6.46 kcal/mol whereas the standard positive drug showed minimum binding energy of −4.92 kcal/mol.

Docking result between astaxanthin and 4LQM apoptotic protein revealed that the interaction occurs with glu804 and his804 by polar interaction which exhibit minimum binding energy of −6.60 kcal/mol. Astaxanthin also interacted with 4LQM protein by forming two hydrophobic bonds (leu718, phe723) (Table 7). On the other hand, the interaction between sorafenib and protein 4LQM showed minimum binding energy of −6.91 kcal/mol by hydrogen bonding and polar interaction with the residues with asn842 and asp855 (Table 8).

Different interactions of astaxanthin and sorafenib with apoptotic protein 2W3L were shown in Tables 9 and 10. It was found that docking between astaxanthin and 2W3L involves polar and hydrophobic interaction with his143, ala90, val101 and try139 residues. But, the mode of docking between sorafenib and 2W3L produces polar (his143), hydrophobic (ala90, val101, try139) and halogen bonding (trp135, glu138).

The minimum binding energy of −5.35 kcal/mol was produced by astaxanthin and 1GFW whereas sorafenib and 1GFW produces −5.03 kcal/mol of least binding energy. A polar and hydrophobic interaction takes place during the docking of astaxanthin with 1GFW protein. The residues asp146, gln161 are involved in polar interaction

Table 4
Molecular docking results of Astaxanthin and Sorafenib with Apoptotic Proteins of Hepatocellular Carcinoma.

Docking	Est. Free Energy of Binding	Est. Inhibition Constant Ki	vdW + Hbond + desolv Energy	Electrostatic Energy	Total Intermolecular Energy	Frequency	Interact. Surface
Astaxanthin + 2OH4	−6.46 kcal/mol	18.54 μM	−9.61 kcal/mol	+0.02 kcal/mol	−9.59 kcal/mol	10%	989.319
Astaxanthin + 4LQM	−6.60 kcal/mol	14.41 μM	−9.66 kcal/mol	−0.09 kcal/mol	−9.75 kcal/mol	10%	1360.8
Astaxanthin + 2W3L	−4.75 kcal/mol	331.12 μM	−7.32 kcal/mol	+0.01 kcal/mol	−7.31 kcal/mol	10%	907.793
Astaxanthin + 1GFW	−5.35 kcal/mol	119.33 μM	−8.79 kcal/mol	+0.02 kcal/mol	−8.77 kcal/mol	10%	980.899
Astaxanthin + 2AR9	−4.62 kcal/mol	411.78 μM	−8.26 kcal/mol	−0.03 kcal/mol	−8.29 kcal/mol	10%	874.206
Sorafenib + 2OH4	−4.92 kcal/mol	249.01 μM	−7.12 kcal/mol	−0.06 kcal/mol	−7.18 kcal/mol	10%	831.538
Sorafenib + 4LQM	−6.91 kcal/mol	8.59 μM	−8.12 kcal/mol	−0.11 kcal/mol	−8.33 kcal/mol	10%	926.312
Sorafenib + 2W3L	−5.53 kcal/mol	88.27 μM	−7.77 kcal/mol	−0.07 kcal/mol	−7.84 kcal//mol	10%	816.731
Sorafenib + 1GFW	−5.03 kcal/mol	206.59 μM	−6.45 kcal/mol	−0.10 kcal/mol	−6.55 kcal/mol	10%	658.429
Sorafenib + 2AR9	−4.68 kcal/mol	372.27 μM	−6.16 kcal/mol	+0.01 kcal/mol	−6.17 kcal/mol	10%	647.171

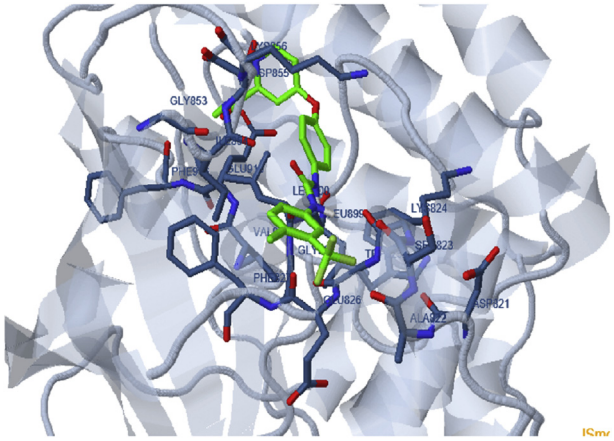

Fig. 2. Molecular docking of Sorafenib and 2OH4.

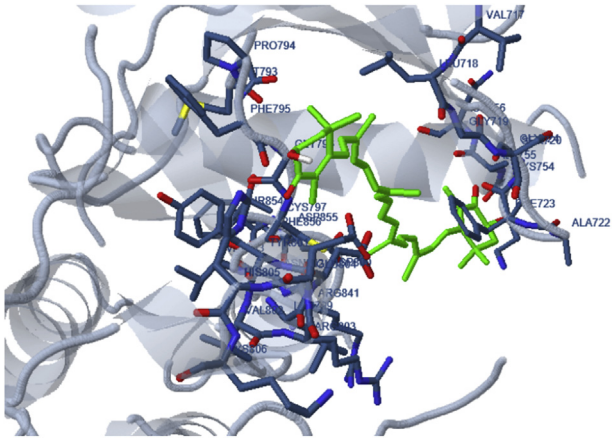

Fig. 3. Molecular docking of Astaxanthin and 4LQM.

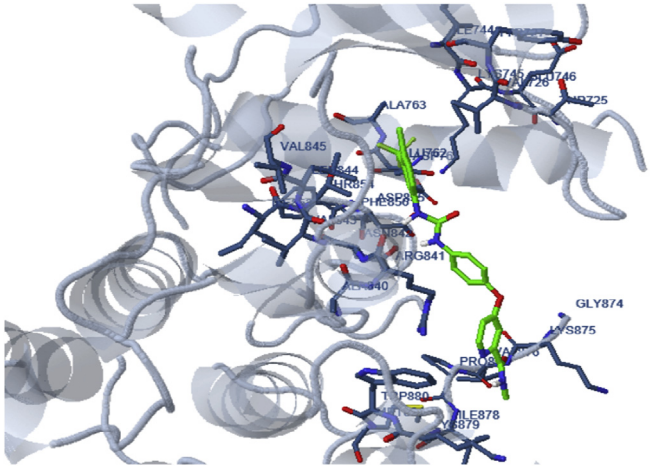

Fig. 4. Molecular docking of Sorafenib and 4LQM.

and phe158, ILE160 are involved in hydrophobic bonding (Table 11). Similarly, the docking result of sorafenib and 1GFW shows hydrogen, polar, halogen and hydrophobic bonding which involves the residues like phe158, tyr83, leu81, leu157, ile159 and ile160 (Table 12).

The docking analysis was carried out between astaxanthin and 2AR9 and the results were tabulated in Table 13. The minimum binding energy between these two compounds was −4.62 kcal/mol. The results showed that the seven hydrophobic bonds of 2AR9 was interacted with astaxanthin compound that involves phe348, phe351, ile396, tyr397,

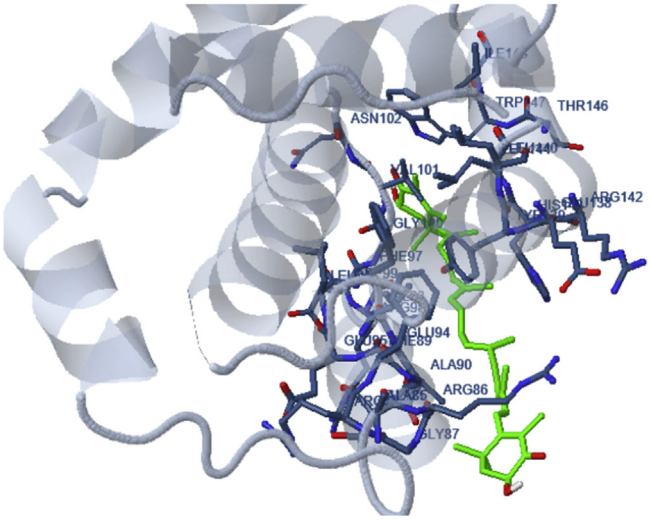

Fig. 5. Molecular docking of Astaxanthin and 2W3L.

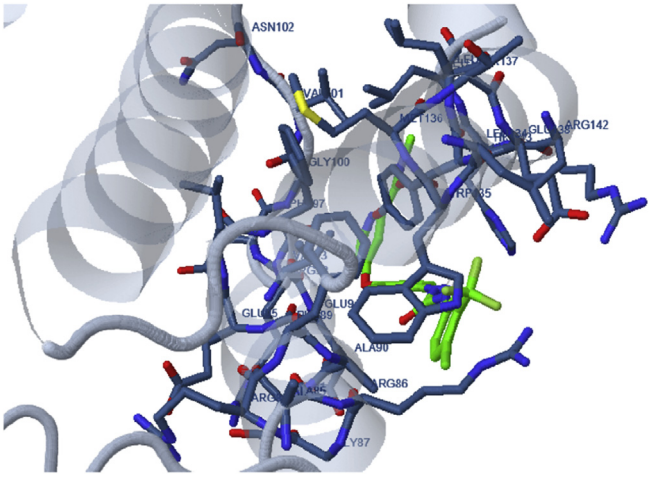

Fig. 6. Molecular docking of Sorafenib and 2W3L.

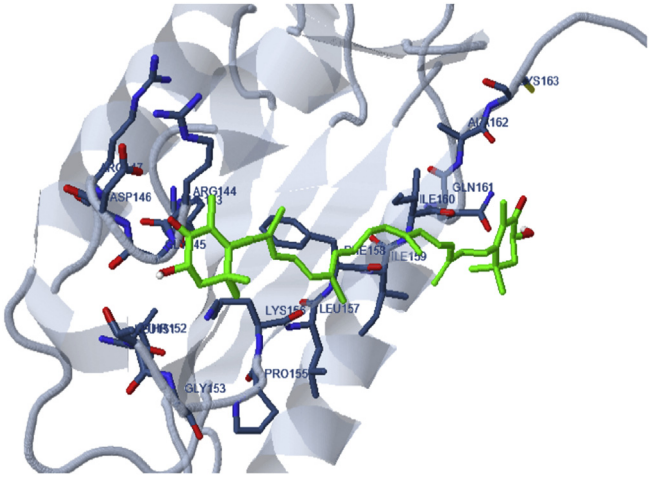

Fig. 7. Molecular docking of Astaxanthin and 1GFW.

met400, cys402, ile403 residues (Table 13). The binding mechanism of sorafenib with 2AR9 was studied using molecular docking method. The docking results show that sorafenib forms hydrogen bonds with ser175, polar bonds with arg178, hydrophobic bonds with leu177 and halogen bonds with asp254, gly255 of 2AR9 receptor (Table 14).

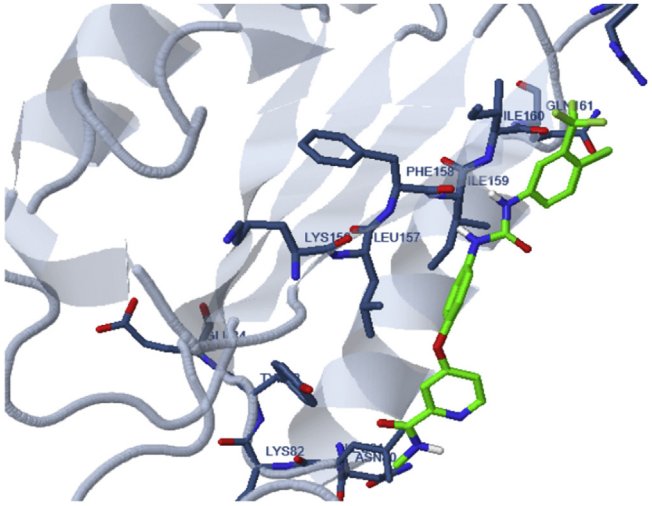

Fig. 8. Molecular docking of Sorafenib and 1GFW.

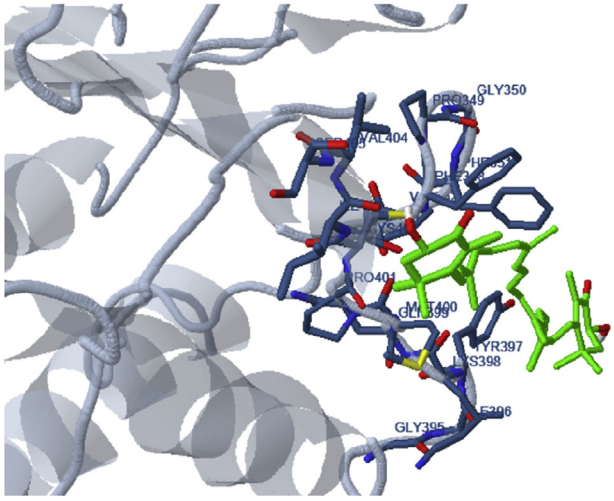

Fig. 9. Molecular docking of Astaxanthin and 2AR9.

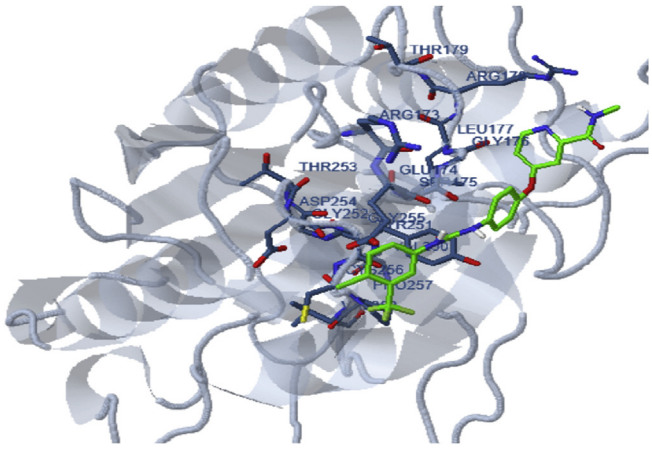

Fig. 10. Molecular docking of Sorafenib and 2AR9.

4. Discussion

In silico methods are extremely useful for both finding potential binding sites and also to discover new molecules that could bind to a known site. Virtual screening and blind docking are often employed in an attempt to discover new medicines. Only few research works was

Table 5
Interaction table for Astaxanthin and 2OH4 (VEGFR-2).

Hydrophobic
C19 – LEU834
C3 – PRO837
C31 – MET867
C29 – MET867
C27 – MET867
C40 – LEU868
C38 – LEU868
C6 – HIS874
C14 – HIS874
C20 – HIS874
C12 – HIS874
C4 – HIS874
C32 – HIS877
C26 – HIS877
C39 – PRO909
C40 – PRO909
C37 – PRO909

Table 6
Interaction table for Sorafenib and 2OH4 (VEGFR-2).

Hydrogen bonds	Polar	Hydrophobic	Pi-pi	Halogen bond
N2 – LYS824	O3 – ASP855	C20 – LEU900	C9 – TRP825	F3 – ALA822
N1 – LYS824		C21 – LEU900		F3 – SER823
N3 – ASP855		C17 – LEU900		F1 – GLU826
N2 – LEU900		C16 – LEU900		

Table 7
Interaction table for Astaxanthin and 4LQM (EGFR).

Polar	Hydrophobic	Cation-pi
O4 – GLU804	C26 – LEU718	H2 – HIS805
O4 – HIS805	C17 – PHE723	
O2 – HIS805	C5 – PHE723	
	C10 – PHE723	
	C27 – PHE723	
	C37 – PHE723	
	C31 – PHE723	
	C33 – PHE723	

Table 8
Interaction table for Sorafenib and 4LQM (EGFR).

Hydrogen bonds	Polar	Halogen bond
N2 – ASN842	H1 – ASN842	F3 – GLU762
N1 – ASN842	H2 – ASN842	F2 – THR854
N2 – ASP855	H2 – ASP855	F3 – ASP855
N1 – ASP855	O2 – ASP855	
	H1 – ASP855	

Table 9
Interaction table for Astaxanthin and 2W3L (Bcl-2).

Polar	Hydrophobic
O4 – ARG86	C24 – ALA90
	C22 – ALA90
	C18 – ALA90
	C12 – ALA90
	C29 – PHE97
	C29 – VAL101
	C27 – VAL101
	C23 – VAL101
	C39 – TRY139
	C35 – HIS143
	C36 – HIS143
	C19 – TPR147

Table 10
Interaction table for Sorafenib and 2W3L (Bcl-2).

Polar	Hydrophobic	Pi-pi	Cation-pi	Halogen bond
H1 – HIS143 H2 – HIS143 N2 – HIS143	C5 – ALA90 C10 – ALA90 C7 – ALA90 C20 – VAL101 C21 – VAL101 C13 – TRY139	C4 – TYR139	H1 – TYR139 H2 – TYR139 H1 – HIS143 H2 – HIS143	F2 – TRP135 F2 – GLU138 F3 – GLU138

Table 11
Interaction table for Astaxanthin and 1GFW (Caspase 3).

Polar	Hydrophobic
O4 – ASP146 O1 – GLN161 H1 – GLN161	C40 – PHE158 C31 – ILE160

Table 12
Interaction table for Sorafenib and 1GFW (Caspase 3).

Hydrogen bonds	Polar	Hydrophobic	Halogen-bond
N2 – PHE158 N1 – PHE158	O3 – TYR83	C21 – LEU81 C6 – LEU157 C12 – LEU157 C15 – LEU157 C16 – LEU157 C11 – LEU157 C4 – ILE159 C9 – ILE159 C13 – ILE159 C5 – ILE159 C2 – ILE160 C3 – ILE160	C11 – ILE160 F3 – ILE160

Table 13
Interaction table for Astaxanthin and 2AR9 (Casapse 9).

Hydrophobic
C33 – PHE348
C31 – PHE348
C29 – PHE348
C27 – PHE348
C35 – PHE348
C40 – PHE351
C38 – PHE351
C39 – PHE351
C35 – PHE351
C11 – ILE396
C20 – TYR397
C25 – MET400
C27 – CYS402
C10 – ILE403
C3 – ILE403

Table 14
Interaction table for Sorafenib and 2AR9 (Caspase 9).

Hydrogen bonds	Polar	Hydrophobic	Halogen-bond
N2 – SER175 N1 – SER175	N3 – ARG178 H3 – ARG178 N4 – ARG178	C4 – LEU177 C6 – LEU177 C8 – LEU177 C9 – LEU177 C11 – LEU177 C12 – LEU177 C18 – LEU177	Cl1 – ASP254 F1 – GLY255

carried out in *in silico* molecular docking of astaxanthin. In present study, sorafenib which was used to treat hepatocellular carcinoma was taken as positive drug. Our finding shows good docking capacity by astaxanthin with five different apoptotic proteins such as VEGFR, EGFR, Bcl-2, Caspase 3 and Caspase 9 than the positive drug sorafenib. The binding mode of astaxanthin with amino acids of varied chemical entity of the target proteins proved the difference in folding of target polypeptide chains upon interaction with the drugs. This study compared *insilico* molecular docking of astaxanthin and sorafenib with different apoptotic proteins performed by different researchers.

According to Guttula et al. (2011), astaxanthin and beta carotene was docked with protein BDNF (Brain derived neurotrophic factor) and RHOD (Ras homolog). BDNF and Astaxanthin-Docking Energy range: Emin = −225.39, Emax = −74.12, BDNF and β-carotene- Docking Energy range: Emin = −220.68, Emax = −69.21, RHOD and Astaxanthin Docking Energy range: Emin = −247.72, Emax = −88.39, Rhod and β-carotene Docking Energy range: Emin = −232.07, Emax = −86.55. In docking the lowest minimum energy has the highest affinity. It is concluded that astaxanthin docking score when compared with β-carotene is lowest so, it has the highest affinity with the target proteins.

The docking was conducted for Osthol Ritter product and sorafenib on BRAF V599E mutant protein. Among the ten compounds derived, docking result shows that Osthol Ritter product (ORP) possess more binding energy. To confirm this Pavithra et al. (2014) have performed induced docking of ORP with the existing drug Sorafenib, both the compounds shows interaction with ASP 593 and GLU 500 and ORP's Glide score is similar to Sorafenib. Hence, they suggest that, the osthol ritter product can be used as a drug candidate against cancer.

The two series of sorafenib derivatives bearing sulfonylurea scaffold were designed and synthesized. The entire target compounds were evaluated the activity against four cancer cell lines and VEGFR2/KDR kinase. Six of the target compounds showed moderate activity and compounds **6c** and **6f** were better. The first series with no substitution in the phenoxy group showed more activity than the second series. Different substitutions of the aryl group affected the cytotoxicity of target compounds. Small halogen atom substitutions of the aryl group contributed to the activity of the first series, while there is no significant regularity of the second series. Although all of the target compounds showed less activity than the positive compounds, structure-activity relationships (SARs) and docking studies indicated that sulfonylurea unit is important to the activity of this kind of compounds. Thus, the results of Wu et al. (2015), suggested that the sulfonylurea sorafenib analogs are worthy of further study. More compounds of sorafenib analogs bearing a sulfonylurea may be screened by replacing the aryl groups by heterocyclic rings in our further study.

In silico studies have been performed for the biological activity of astaxanthin which is the reddish food colorant. Molecular docking experiments have shown that astaxanthin can bind to the monoamine oxidase A (MAO-A) active centre with predicted Kd = 4.4 × 10-6 M. Distinguishly, monoamine oxidase B (MAO-B) does not, practically, bind astaxanthin; the very negligible binding with predicted Kd > 109 M might occur on the enzyme surface apart from its active centre. Hence, astaxanthin may act as a selective and reversible MAO-A inhibitor. Selective and reversible MAO-A inhibitors are widely used as antidepressant and anxiolytic drugs whereas non-selective and irreversible inhibitors have been withdrawn due to toxic side effects caused by their interactions with other drugs and some food products (Safarova et al., 2016).

Sahrawat and Chawla (2016) investigation was designed to identify the potential off-targets of Sorafenib which could be responsible for its reported undesirable side effects. Molecular docking was used to test the efficacy of structural analogs of Sorafenib against B-Raf using FlexX and it was found that the analog with CID:10151557 had a high potency with minimum number of clashes, low lipophilic score and high match score, similar to Sorafenib. To identify the potential off-target/s

of Sorafenib, macromolecular surface similarity detection software MEDIT SA MED-SuMo was used and the results obtained were validated through literature. The possible off-targets obtained belonged to the family of protein tyrosine kinases i.e. VEGFR-2, VEGFR-3, platelet-derived growth factor receptor beta, Flt-3, and c-KIT, each of which were docked with Sorafenib. Based on high docking scores and similarity with B-Raf for its binding site interacting residues, it was concluded that vascular endothelial growth factor tyrosine kinase receptor (VEGFR) is a potential off-target of anti-cancer chemotherapeutic agent Sorafenib.

The *in silico* Molecular docking studies on the phyto constituent of Marine Bioactive Astaxanthin derivative, CID 05281539 clearly demonstrates that it potentially inhibited the Tau protein hyper phosphorylation activity. The hydrogen bond interaction plays a key role to predict the amino acid residues presented in between selected target protein region and ligand molecules. The hydrogen bond interaction region through Pymol software showed a group of polar residues such as ASN-689, ASN-686, ASP-700, LYS-585, ILE-562, ARG-96, ARG-180, GLU-97, GLN-89, GLN-795 etc. located on the binding cavity of GSK-3β (1J1B). The overall results summarized from various insilico analyses suggested that the compound CID 05281539 is strongly involved in inhibition of Tau hyper phosphorylation with reference to Alzheimer's Disease (Praveen and Yellamma, 2017).

5. Conclusion

The molecular docking studies between human growth factors and apoptotic proteins with both Astaxanthin and Sorafenib clearly demonstrated the mode of binding and interacting active site amino acids between them. Since astaxanthin was found to bind the apoptotic proteins with least free energy less than compared to positive drug, it may probably activate apoptotic proteins in hepatocellular carcinoma thereby acting as potent anticancer agent. Thus, based on previous reports, our study also shows good binding capacity of astaxanthin when compared with the positive drug sorafenib. The Astaxanthin and its derivatives can be effectively studied against different apoptotic and receptor proteins Hence, further investigation has to be carried out and to be confirmed by *in vivo* studies using mice model.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bcab.2019.101076.

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