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Bixin-a Diapocarotenoid as Inhibitor of Glutathione Peroxidise (GP×1): A Potential Target for Cancer Treatment: An *in silico* Study

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ABSTRACT: Cancer is a significant health problem and leading cause of deaths worldwide. These cells are gaining resistance against different chemotherapeutic agents. Hence, exploring new targets is a continuous process. Cancer cells produce greater amount of reactive oxygen species (ROS) and for their progression and metastasis upregulation of antioxidant enzymes are required. Glutathione peroxidase (GPX) is a selenocystic –containing peroxidase enzyme which defends mammalian cells against oxidative damage by reducing hydrogen peroxides and organic hydroperoxides. The enzyme GP×1 is over expressed in various cancer cells what eliminates reactive oxygen species (ROS), weakens apoptosis and induce drug resistance promoting cancer cell survival. Exploring GP×1 inhibitors and their applications for cancer therapy is mandatory. Hence, in the present investigation GP×1 inhibition potentials of the Red pigment Bixin was investigated *In silico*. An advanced autodock 4.2 version was used for the study. Bixin has shown strong inhibition potentials of GP×1compared to the standard drug. Furter, DNA binding studies were also performed and strong binding of Bixin to the DNA was recorded compared to the standard drug -5-Fluoro Uracil.

Hence, from the binding scores, hydrogen bond interactions with the receptors, it can be concluded that the compound Bixin may be developed as a drug for treating cancers which inhibits GP×1 enzyme. However, further *in vitro* and *in vivo* investigations are required to develop Bixin as a final drug.

Keywords: In silico, GP×1, Bixin, Autodock, cancer, DNA binding.

INTRODUCTION

Cancer results from loss of cell cycle control and is associated with abnormal cell growth (Sumitra and Krunal 2013). Cancer these days is a leading cause of deaths worldwide with the most common cases being colon, lung, and rectum, stomach, liver, and breast (Nurcahyanti *et al.*, 2021). Deaths with cancer isa significant health problem worldwide due to lack of early detection methods and combating it is one of the greatest challenges to mankind. Cells generate reactive oxygen species (ROS) in normal oxidative respiration whereas, cancer cells produce greater amount of ROS due to enhanced metabolism and mitochondrial dysfunction (Nogueira and Hay 2013). Reactive oxygen species (ROS) plays a key role in maintaining cellular signal transduction and homeostasis at physiological levels, and increased ROS levels which damage to cellular structure and function called oxidative stress (Sies and Jones 2020). Glutathione peroxidise (GPx) family contain oxidative enzymes which protect the organism from damage by reducing hydroperoxides to their corresponding alcohols and free hydrogen peroxide to water (Bakan *et al.,* 2003). Cancer cells attain resistance to chemotherapy due to increased ROS levels which are countered by increased activity of antioxidant enzymes in order to avoid detrimental effects of oxidative stress (Lee *et al.*, 2017). Among different types of glutathione peroxide enzymes reported GPx1 is a vital enzyme preventing the harmful build up of intracellular hydrogen peroxide (Lubos *et al.*, 2011). Hence, for tumor progression and metastasis, cancer cells require oxidant scavenging and the upregulation of antioxidant enzyme expression (Chang *et al.*, 2020). Despite development of different therapeutic approaches, the number of cancer deaths are increasing day by day with the development of resistance against anti-cancer drugs. Hence, search for alternate targets has become mandatory. Thus, the development of GP×1 inhibitors could offer a promising avenue to novel anticancer drugs.

Bixin is a liposoluble diapocarotenoid (red pigment) produced by a herb Annatto (*Bixa orellana*) which is popularly called Sinduri or lipstick tree. Bixin is the methyl ester of dicarboxylic acid norbixin (Scotter, 2009) which is mainly present in seeds which accounts for nearly 80% of total carotenoids present in the seed (Islam *et al.*, 2014). Multiple medicinal properties of

bixin *viz.,* antioxidant, anti-inflammatory, antiasthma and neuroprotective (Qiu *et al*., 2022) antibacterial, antifungal properties of Bixin leaf extracts were reported by Stohs (2014) and seed extracts by Fleischer *et al.* (2003). Bixin is also used mostly in dairy industry, textile industry, cosmetic industry and also used as animal feed.

Hence, owing to the medicinal properties, of Bixin, in the present investigation glutathione peroxidise (GPX1) inhibition potentials and DNA binding potentials were studied *in silico.*

MATERIALS AND METHODS

Preparation of Ligands: In the present investigation, the red pigment Bixin and the standard drug mercaptosuccinicacid used as ligands were drawn using Chem Draw software and saved in .mol format. Later, the ligands in mol format were converted to 3D PDB format using Accelrys Discovery Studio 2.3. Hydrogen atoms were added and energy minimisation was done and saved as pdbqt file using Autodock software.

Preparation of target Proteins: Molecular docking of Bixin and the standard drug mercaptosuccinic was performed with receptor glutathione peroxidise (GPx1) using Auto dock software, an interactive molecular graphics programme to understand the protein-ligand interactions (available from http://viba.scripps.edu/). The crystal structures of GPx1 (PDB ID: 1GP1) with resolution of 2.0°A was obtained from PDB data base (http://www.rcsb.org./pdb). The bound ligands, hetero atoms, water molecules were removed, and polar hydrogen atoms were then added, Kollman charges and salvation parameters were assigned by default using Auto dock software. Similarly, crystal structure of DNA was down loaded from protein data bank with resolution of 1.90°A and processed as described above.

Validation of Software: Before performing docking the Auto dock software was validated by downloading the X-ray crystal structure of the receptors glutathione peroxidise (PDB I D: 1GP1) from protein data bank and the co crystallized ligand was redocked so as to reproduce the original interactions of the reference protein-ligand complexes comparing the root-mean square distance of the experimentally determined pose with the docked pose.

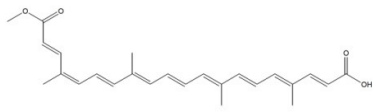
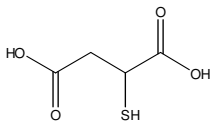
In silico studies: Virtual screening for interaction of Bixin and mercaptosuccinic acid with glutathione peroxidise (GPX1) and DNA by molecular docking

Virtual screening for interaction of ligands Bixin and the standard drug mercaptosuccinic with receptor GPx1 (PDB I D: 1GP1) and DNA (PDB ID: 1BNA) was performed by molecular docking. After preparing the ligands as well as receptors, both were converted into the pdbqt format using the automated docking tool Auto Dock which was later used for docking. For GPX1 a grid box was prepared to cover the pocket and the main residues of protein binding site with the grid size of X = 40, Y = 40, and Z = 40. The coordinates used for docking the ligands with 1GP1 were x=34.057; y=62.084; z=26.267. Similarly, a grid box was also prepared for DNA with the grid size of X=40, Y=40, and Z=40. The coordinates used for docking the ligands with DNA were =15.1648; y=21.7027; z=9.0633. An advanced molecular docking program Auto Dock vina, version 1.1.2 available from http://vina.scripps.edu/download.html was used for docking against the receptor to estimate the binding affinities (kcal mol^{-1}). The ligands were evaluated *in silico* against glutathione peroxidase (PDB ID: 1GP1) and DNA (PDB ID: 1BNA) in triplicates and based on complete docking search (ten runs) the average of the best conformation was chosen with the lowest docked energy, The interaction of glutathione peroxidase with the ligands, hydrogen bonds, bond lengths and Root Mean Square Difference (RMSD) was analyzed using PyMOL software (http://pymol.sourceforge.net/).

RESULTS AND DISCUSSION

In the present investigation, glutathione peroxidise inhibition potentials of the red pigment Bixin was studied using mercaptosuccinic acid as the standard reference drug (Table 1).

Table 1: Structure and IUPAC names of bixin and standard drug.

Sr. No.	Name of the Compound	IUPAC Name	Molecular Structure
1.	Mercapto succinic acid	2-sulfanylbutanedioic acid	
2.	Bixin	2*E*,4*E*,6*E*,8*E*,10*E*,12*E*,14*E*,16*Z*,18*E*)-20-methoxy-4,8,13,17-tetramethyl-20-oxoicosa-2,4,6,8,10,12,14,16,18-nonaenoic acid	

Further, DNA binding activity was also studied *In silico* using computational approaches which is very useful for understanding organic compounds and their interactions with the drug targets. These studies can be used as preliminary investigation in designing ligands and studying their interaction with the target proteins before proceeding to wet lab. Autodock software was used for this docking studies 4.2.6

In silico studies of Bixin with receptor GP×1: Molecular docking of test compound Bixin into the active site of GP×1 was found to be very successful based on the complex formation of GP×1 with the ligand Bixin. The Binding energy, hydrogen bond interactions, bond length, RMSD, active site residues and orientation of the docked compounds in the active site were visualized. The test compound screened showed the best RMSD value of 0.000, indicating statistically considerable interaction. The negative and low value of ΔG indicated a strong favourable bonding between GP×1 and the ligand in their most favourable conformations. The binding energy recorded was -7.1 indicating a relatively higher interaction of test compounds Bixin with GPx1 compared with the standard reference drug Mercaptosuccinic acid which showed a binding energy 0f -3.7.

In the present study, in order to find the other possible Bixin binding sites, DNA binding studies were also performed *in silico*. The red pigment Bixin was found to bind the DNA with a higher binding score of -6.2 compared to the standard reference drug 5-Fluoro Uracil which showed a binding score of -3.5. The details of binding energy, hydrogen bonds formed and the catalytic site residues involved in the protein-ligand complex of DNA with Bixin and standard drug 5-Fluoro Uracil is depicted in Table 2 and Fig. 1-4.

Table 2: Interacting amino acids, H-bonds, and binding scores of proteins Glutathione peroxidase GP×1 (PDB ID: 1GP1) and DNA (PDB ID: 1BNA) with ligands.

Name of the ligand	Affinity kcal/mol	Number of hydrogen bonds	Interacting Amino Acids
PDB ID: 1GP1			
Bixin	-7.1	04	Threonine-53 Leucine-67 Threonine-53 Glutamine-59
Mercaptosuccinic acid	-3.7	05	Alanine-21 Alanine-21 Leucine-20 Leucine-107 Phenyl Alanine-105
PDB ID: 1BNA			
Bixin	-6.2	04	Adenine-05 Adenine-06 Cytosine-11 Guanine-16
5-Fluoro Uracil	-3.5	07	Guanine-16 Cytosine-11 Cytosine-11 Cytosine-09 Cytosine-09 Guanine-10 Guanine-10

In the present study, Bixin showed strong interaction with different amino acids of glutathione peroxide protein *viz*., Threonine-52, Leucine-67, Threonine-53 and Glutamine-59. Similarly, the standard drug Mercaptosuccinic acid showed interaction with Alanine-21, Leucine-20, Leucine-107 and Phenylalanine-105. In the DNA binding studies performed with Bixin, the binding nitrogen bases recorded were Adenine-15, Cytosine-5,6 and Guanine-16 whereas, the standard 5-fluoro uracil showed interactions with Guanine-16, Cytosine-11,9,Guanine-10,10. For tumor progression and metastasis, cancer cells require oxidant scavenging and upregulation of antioxidant enzyme expression (Chang *et al*., 2020) in which Glutathione peroxide (GP × 1) is the key enzyme which prevents intracellular hydrogen peroxide accumulation (Lubos *et al*., 2011) whose inhibition leads to death of cancer cells. In the present study, Bixin a red pigment produced by plant seeds strongly inhibited the enzyme GPx1 compared to the standard drug. Similarly, Bixin also showed strong binding with DNA whose binding interferes with its replication, transcription and translation which are key for cell proliferation and survival.

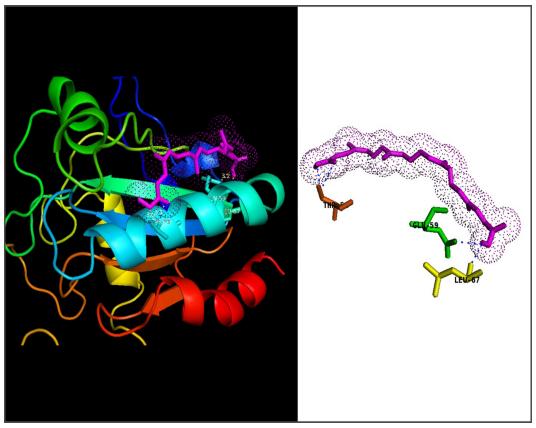


Fig. 1. Snapshot showing docking of glutathione peroxidase (PDB ID: 1GP1) with Bixin.

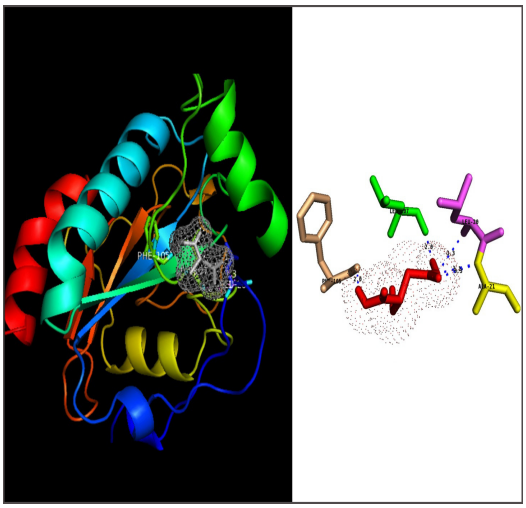


Fig. 2. Snapshot showing docking of glutathione peroxidase (PDB ID: 1GP1) with standarddrug Mercaptosuccinic acid.

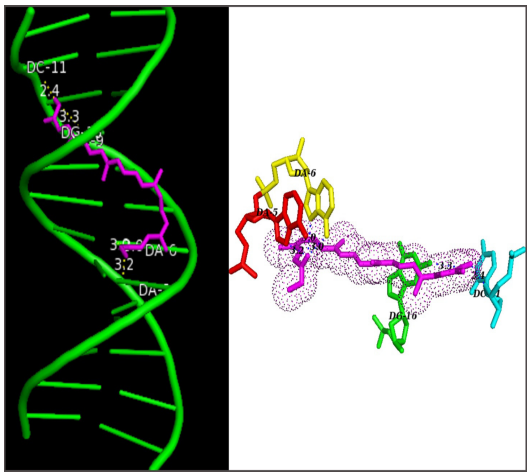


Fig. 3. Snapshot showing docking of Bixin with B-DNA (PDB ID: 1BNA.

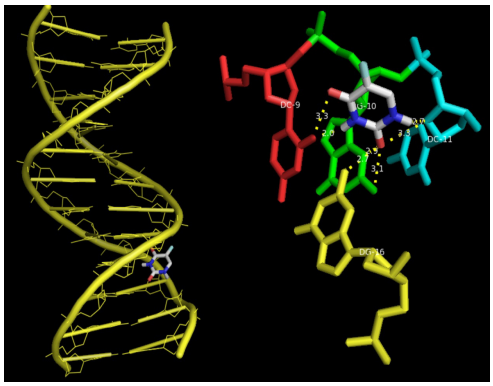


Fig. 4. Snap shot showing docking of standard drug Mercaptosuccinic acid with B-DNA (PDB ID:1BNA).

CONCLUSIONS

In the present investigation, Glutathione peroxide (GP×1), a vital enzyme involved in minimizing oxidative stress to the cancer cells is inhibited by a red pigment produced by plant seeds which leads to death of cancer cells. Similarly, in order to find other possible targets where Bixin binds and prevents cancer cell proliferation DNA binding studies were performed and strong binding of Bixin with DNA was found when compared to the standard drug -5-fluoro uracil. Binding of Bixin to DNA interferes with the protein synthesis and replication leading to death of the cancer cells. However, further investigation in *In vitro* and *in vivo* are required to confirm.

FUTURE SCOPE

Bixin can be developed as an anticancer drug which targets GP×1 enzyme which is up regulated in cancer cells whose inhibition can prevent proliferation of cancer cells.

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Conflicts of Interest. None.

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