Original Article

In Silico Investigation and Structural Interaction of Harmine and Piperine Ligand on Bak in Pancreatic Cancer

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Abstract

Pancreatic cancer is a lethal malignancy that typically affects males over the age of 40 and has a rapid progression. Its occurrence has progressively grown in recent years. It is responsible for 2% of all cancers and 5% of all cancer-related deaths. In this study, we evaluate a possible lead molecule for pancreatic cancer by looking at the activity of harmine and piperine *in-silico*. The compounds were chosen for their diverse nature and potential anti-cancer properties. The Fit score, normalized fit score, and Z^1 score were computed in this study. Protein-ligand interactions provide pharmacophore interaction characteristics. In the molecular scenario, docking is often utilized to study the interaction between the target ligand-receptor and the binding orientation of molecules with their protein receptor. The molecular docking studies made use of Marvin sketch PyRx 0.9. Based on the findings, piperine and harmine have good binding affinities that are equivalent to those of standard paclitaxcel. The ligands' molecules have demonstrated energy minimization of between -6.7 and -8.7 kcal can be utilized to enhance, simulate, and verify *in vitro* and *in vivo* investigations.

Keywords : Pancreatic cancer, Harmine, Piperine, Fit score, PyRx , Paclitaxcel, *Insilico.*

1. INTRODUCTION

The term "cancer" refers to a wide range of disorders defined by the development of aberrant cells outside of their normal borders, which can subsequently spread to other sections of the body and/or other organs. Pancreas is an important retroperitoneal organ with exocrine and endocrine functions. Tumors of pancreas are divided into two groups 1) Non-endocrine pancreas tumors 2) Endocrine pancreas tumors. Pancreatic cancers are genetically well-defined malignancies[(1-2)]. Clinically, pancreatic neoplasms range as benign cysts to fatal tumors. These malignancies also have a wide range of genetic mutations. A malignancy of the pancreas somatic mutations of oncogenes and tumor suppressor genes cause genetic disease . Genes often changed in pancreatic neoplasms have had mutation prevalence studied. Pancreatic Ductal Adenocarcinoma (PDAC) may be inherited in as much as 10% of cases[(3-6)]. Smoking cigarettes may include carcinogens that enter the bloodstream and harm the pancreas, according to researchers. Exocrine pancreatic cancer is thought to be caused by smoking cigarettes. Using smokeless tobacco products, including e-cigarettes and cigars, increases the risk of certain cancers. Pancreatic cancer risk rises with advancing age. About two-thirds of pancreatic cancer patients are within the age of 65. As a result of the high level of tobacco use in males, pancreatic cancer is 30 percent more likely to occur in men than in women[(8-9)]. There is a genetic component to it as well an estimated 227,000 people die each year from pancreatic cancer, making it the fourth most common cancer-related mortality . Especially in industrialized nations, the number of cases is steadily rising. An estimated 39,590 people died in 2014 as a result of pancreatic cancer in the U.S. alone. Pancreatic cancer has a five-year survival rate of fewer than 5% because it is diagnosed at such an advanced stage. It becomes more frequent with age, and males are more likely to suffer from it than women. In 2020 over 495,700 cases of pancreatic cancer were registered, out of which over 466,000 cases have ended in death (over 94%). Which is a higher mortality rate even in comparison to breast cancer with highest number of cases (30% mortality) and lung cancer which has highest number of deaths (81% mortality).

Harmine obtained from Syrian rue (*Peganum harmala*), a perennial plant which can grow to about 2.5 ft tall, but normally it is about 1 ft tall. Variety of pharmacological and therapeutic benefits were seen in the recent years. Analytical studies on the plant's chemical composition reveal that beta- carboline alkaloids such as harmalol, harmaline, and harmine are the most prominent ingredients. Harmine was also used in the Middle East and China for centuries. Harmine has a broad range of biological activities, such as anti-inflammatory, neuroprotective, anti- diabetic, and antitumor. Additionally, harmine does also have insecticidal, antiviral, and antibacterial properties[(9-12)].

Piperine obtained from Black pepper (*Piper nigrum*), a woody climber and may reach heights of 10 metres (33 feet) by means of its aerial roots. Piperine has been demonstrated to have basic effects on p- glycoprotein and several enzyme systems, leading to bio-transformative effects such as chemoprevention, detoxification, and increase of the absorption and bioavailability of herbal and conventional medications. Piperine is shown to have antiproliferative, antitumor, anti-angiogenesis, antioxidant, antidiabetic, anti-obesity, cardioprotective, antimicrobial, antiaging, and immunomodulatory effects in numerous *in-vitro* and *in-vivo* experiments performed[12-14]

2. MATERIALS AND METHODS

2.1 Creation of interactive chemical library

The library for the 124 compounds was prepared. The molecules were chosen based on the heterogeneous nature that are suspected to have anti-cancer property.

2.2 Pharmacophore mapping for identifying a therapeutic target

With a complete target pharmacophore database, online web servers were employed to assess the compounds for possible therapeutic target identification. Following the study of the compounds, the Fit score, normalized fit score, and Z^1 score were calculated. Pharmacophore interaction properties resulting from the protein–ligand combinations were obtained for 12 compounds. A higher score implies that the chemical will be more active against the macromolecules targeted.

2.3 Molecular docking

Docking is commonly used in the molecular scenario to understand the interaction between the target ligand-receptor and the binding orientation of molecules with its protein receptor. Bioinformatics tools have been used to perform the in-silico research. We also used some offline programming, such as the protein data bank (PDB), the PubChem database, and the Marvin sketch PyRx 0.9 was used in the molecular docking studies.

The human (PDB) was prepared using the offline programme protein data bank (PDB), and aresolution of was attained. We removed the protein's crystal water, and added missing hydrogens, protonation, ionization, and energy minimization. The SPDBV (Swiss Protein DataBank Viewer) force field has been used to minimise energy.

The amino acid residues present in the protein's active site were determined using the Protein-ligand interaction profile (PLIP), an offline tool in google. Ligands were prepared by using Marvin sketch tool, and the molecules were designed in two and three dimensions. After designing, the structures were optimised in 3D optimization in Marvin sketch and saved as a pdb format. The docking study was carried out using PyRx 0.9. PyRx is a Python programming language that can run on practically any modern machine, from a PC to a supercomputer. PyRx was usedfor molecular docking and determining the binding affinity of the ligand and protein.

3. RESULTS AND DISCUSSION

3.1 Creation of interactive chemical library

A library of 124 compounds was created. The compounds were chosen for their diverse nature and potential anti-cancer properties. A cursory study of the literature also revealed the presence of anticancer action. Based on their subcellular localization and target protein, the top 12 compounds out of the 124 compounds were selected mentioned in Table 1.

Table 1. Lead molecules and their predicted targets

S.No	Lead Molecule	Target protein	Fit Score	Normalized Fit Score	Z^1 Score	Subcellular localization
1.	Harmine	Proto- oncogene serine/threonine-protein kinase Pim- 1	3	1	1.5914	**Nucleus, Cytoplasm and Cytosol**
2.	2- Methyl Harmine	Caspase-7	3	1	1.6271	**Cytoplasm and Cytosol**
3.	6 MethoxyHarmine	Proto- oncogene serine/threonine-protein kinase Pim- 1	3	1	1.4227	**Nucleus Cytoplasm and Cytosol**
4.	8 Methoxy Harmine	Proto- oncogene serine/threonine-protein kinase Pim-1	3	1	1.5771	**Nucleus Cytoplasm and Cytosol**
5.	Ethenyl ester Harmine	Mitogen-activatedprotein kinase 8	2.9	0.9949	1.3406	**Nucleus Cytoplasm and Cytosol synapse**
6.	Harmine methyl iodide	Caspase-7	3	1	1.5446	**Cytoplasm and Cytosol**
7.	Harmine N oxide	Caspase-7	3	1	1.5424	**Cytoplasm and Cytosol**
8.	Tetrahydro Harmine	Beta- secretase 1	2.9	0.9994	1.0755	**Lysosome**
9.	N Ethyl Harmine	Proto- oncogene serine/threonine-protein kinase Pim- 1	3	1	1.0835	**Nucleus Cytoplasm and Cytosol**
10.	Harmine hydrochloride	Aldo-ketoreductasefamily 1 member C2	3	1	-0.071	**Cytoplasm and Cytosol**
11.	Scopolitine	Cell divisionprotein kinase 2	2.9	0.9907	1.3853	**Cytoskeleton Cytoplasmand Cytosol Nucleus Endosome**
12.	**Scoparone**	**Carbonic anhydrase 2**	**2.9**	**0.9764**	**1.4008**	**Plasma membrane Cytoplasm and Cytosol**

3.2 Pharmacophore mapping for identifying a therapeutic target

Using pharma mapper, pharmacophore mapping was done, and two compounds Piperine and Harmine were revealed to interact with cancer-related proteins based on the fit score, normalized fit score, and z^1 score. Based on the interactions with the proteins the following compounds were selected Harmineand Piperine. Piperine and harmine's hydrophobic interaction and hydrogen acceptor properties were also identified. The Pharmacophore score for Piperine and Harmine are revealed in (Table 2 and Table 3)

Table 2. Pharmacophore score for Piperine

Pharmacophore Color Scheme					
Hydrophobic	Positive	Negative	Donor	Acceptor	Aromatic
2	0	0	0	1	0

Figure 1. Pharmacophore model of Piperine

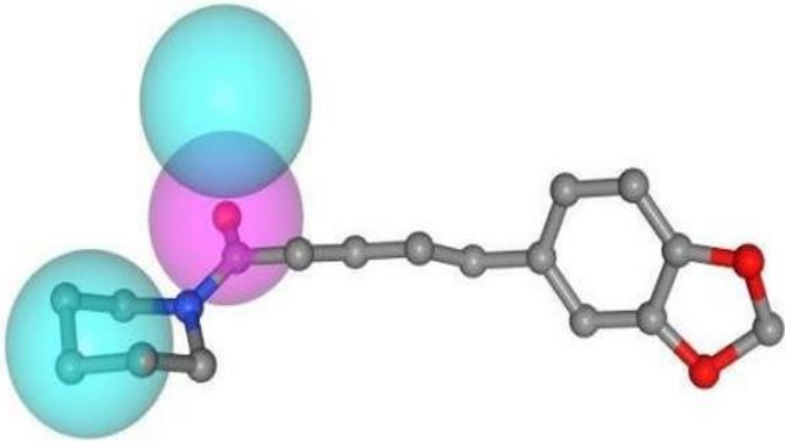


Table 3. Pharmacophore score for Harmine

Pharmacophore Color Scheme					
Hydrophobic	Positive	Negative	Donor	Acceptor	Aromatic
3	0	0	0	0	0

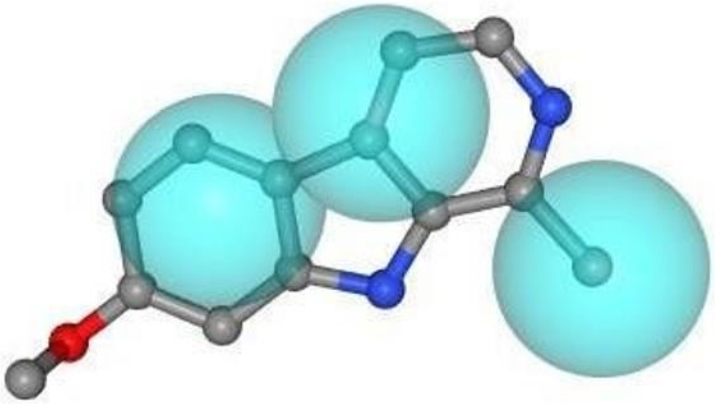


Figure 2. Pharmacophore model of Harmine

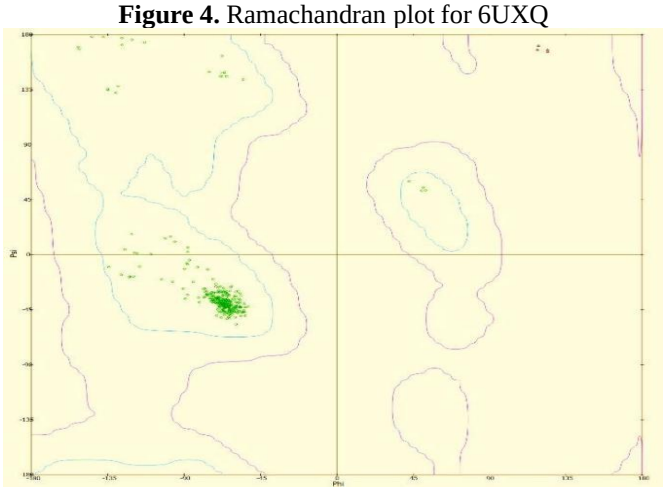
3.3 *In-silico* screening

3.3.1 Preparation of protein and identification of active site

The 3D structure of the protein human BAK (Bcl-2 homologous antagonist/killer) wascollected from the protein data bank (PDB: **6UXQ**) (**Figure 3.)**. The active site amino acid residues were identified from the protein ligand interaction profile (PLIP). The Prepared protein was validated by utilizing the Ramachandran plot as shown in (Fig 4)

Figure 3. Protein structure of 6UXQ

Figure 4. Ramachandran plot for 6UXQ



3.3.2 Molecular docking

In PyRx, binding affinity parameters were considered for selecting the best "HITS" and compared with the known anticancer agent Paclitaxcil. PyRx binding energy is the energy of the interaction between the protein and the ligand. This value strongly indicates the extent of interaction of proteins and ligands. The ligands' binding affinity is shown in **(Table 4)**,indicating that the compounds were effectively bound to the active site of BAK (Bcl-2homologous antagonist/killer). The bonds and Pi-Pi interactions of BAK and these compoundswere analysed.

The piperine molecule showed the highest binding affinity with BAK (-8.8) and BAK protein piperine complex made zero conventional-hydrogen bonding, nine hydrophobic interactions (GLY122, ILE123, ASN124, ASN124, TRP125, VAL129, GLY133, LEU132, LEU132), one pi-sigma interactions (VAL128) and one pi-pi stacked interactions (TYR136). The 2D and 3D interactions are mentioned in (**Fig 5 and Fig 6**)

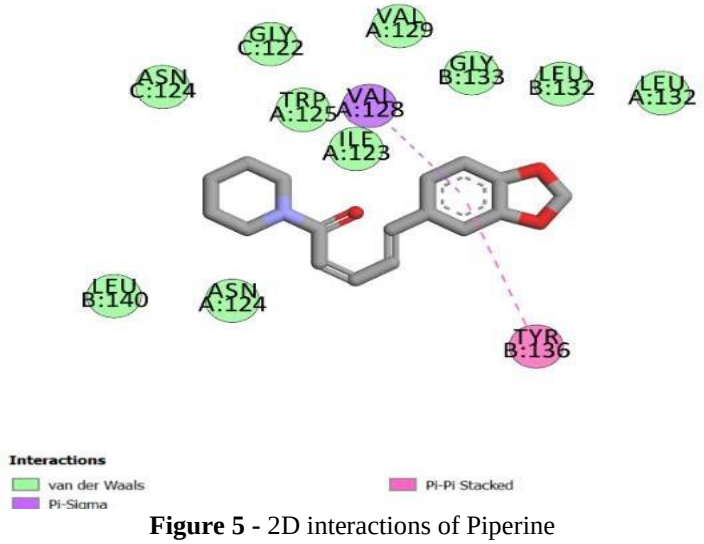


Figure 5 - 2D interactions of Piperine

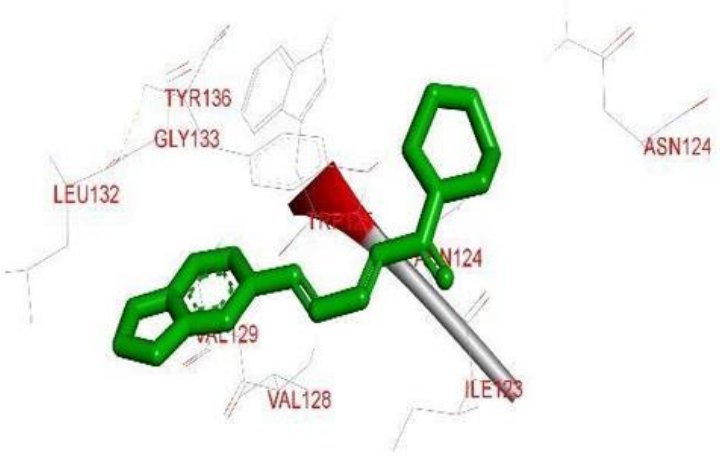


Figure 6 - 3D interactions of Piperine

The harmine molecule showed the binding affinity with BAK (-6.9) and BAK protein- harmine complex made one conventional-hydrogen bonding (SER121), 13 hydrophobic interactions (ASP83, ARG87, ASP90, GLY122, ILE123, ILE123, ASN124, ASN124, TRP125,ARG127, VAL128, TYR136. ARG137) and one pi-alkyl interactions (SER140).

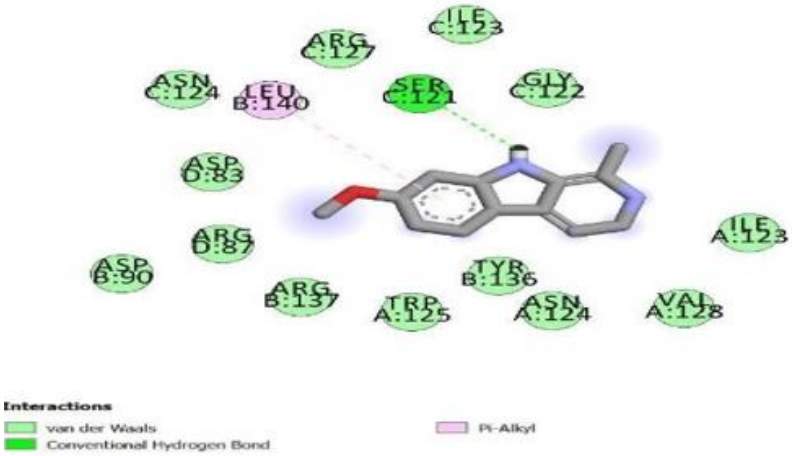


Figure 7 - 2D interactions of Harmine

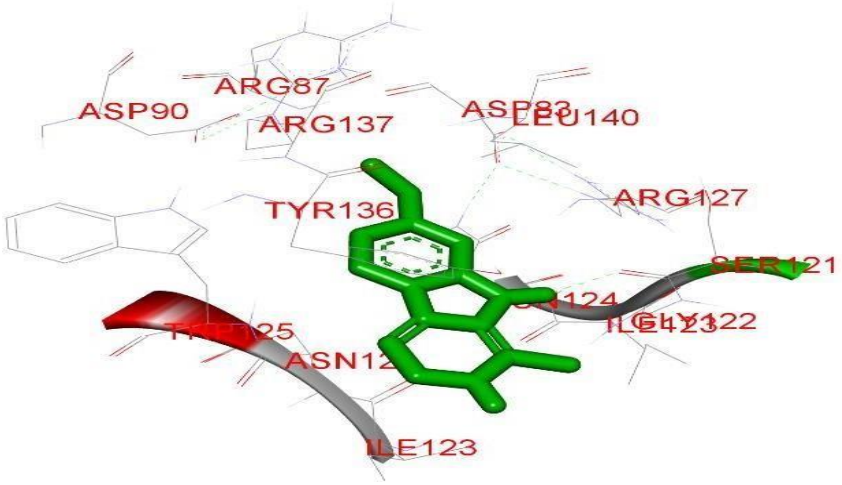


Figure 8 - 3D interactions of Harmine

The paclitaxcel molecule showed the binding affinity with BAK (-7.5) and BAK protein-harmine complex made zero conventional-hydrogen bonding, six hydrophobic interactions (TRP125, VAL129, LEU132, GLY133, GLY135, TYR143), one pi-sigma interactions (VAL128), three pi-pi stacked interactions (TYR108, PHE111, TYR136) and three pi-alkyl interactions (ALA107, LEU138, ALA139).

Figure 9- 2D interactions of Paclitaxel

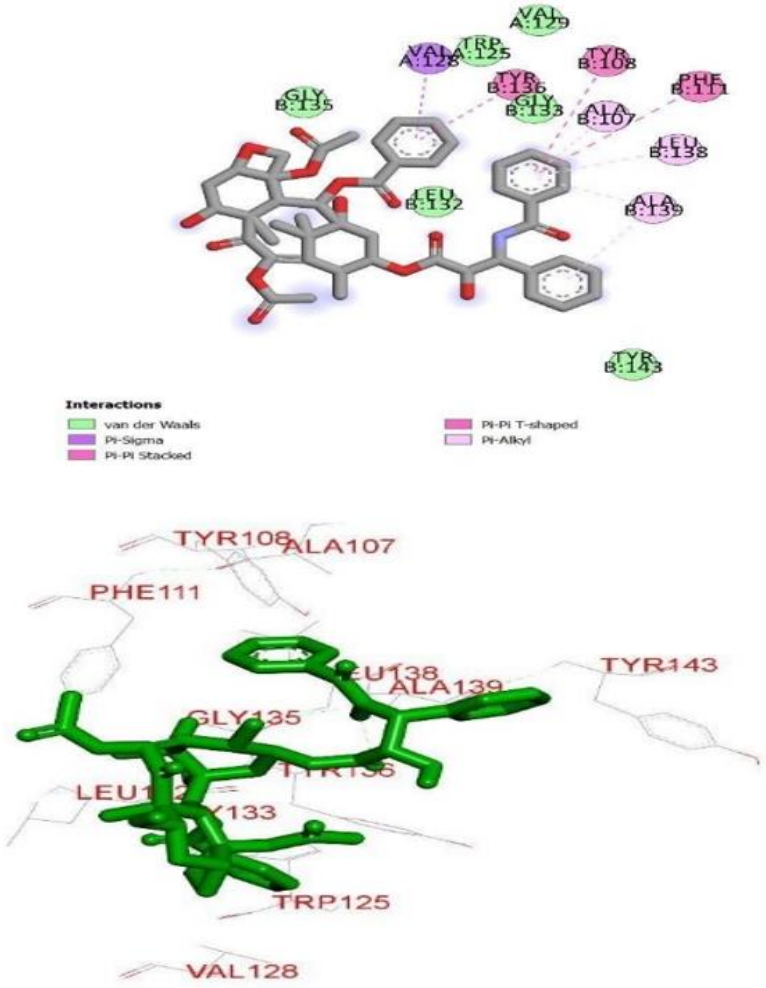
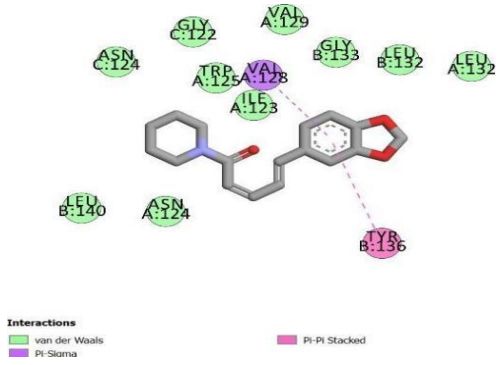
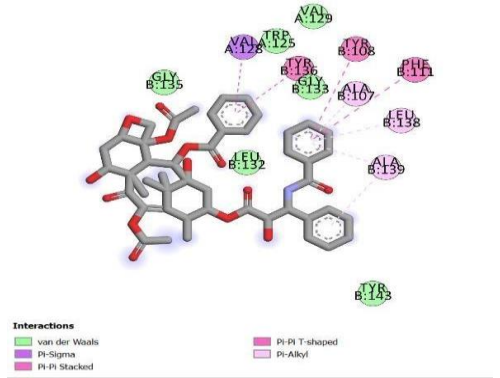
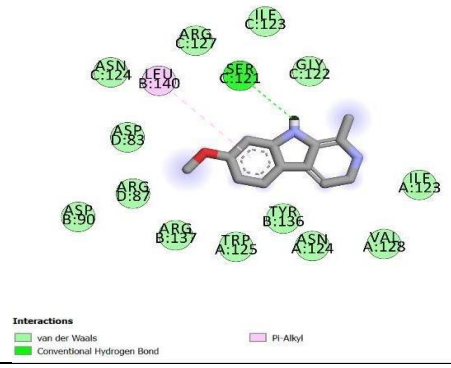


Figure 10 - 3D interactions of Paclitaxel

Table 4. Binding affinity and 2D interactions of ligands

Ligand	Binding affinity	2D interactions
Piperine	-8.8	
Paclitaxel	-7.5	

Harmine	-6.9	

4. CONCLUSION

The present study computed the binding energy and binding site to that of our target receptor, the BAK (Bcl-2 homologous antagonist/killer) . BAK was docked with the ligands harmine and piperine in the current work. The present study's findings indicated a favorable binding site and good docking value coupled with electrostatic, Vander Waals forces of attraction, Pi-Pi bonds , Pi-Sigma bonds and desolvation energies, all of which are crucial for binding. These elements are considered while creating novel BAK inhibitors. These *insilico* investigations demonstrated a decent binding energy value, with BAK values between -6.9 Kcal and -8.8 Kcal, which is acceptable and comparable with that of the standard paclitaxel which is currently used in pancreatic cancer treatment . The substantial interactions of residues with the binding site were also noticed in addition to docking scores. The *in silico* research can be utilised to create new pancreatic cancer targets and study their mechanisms through *in vitro* and *in vivo* studies.

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6. Conflicts of Interest: The authors declare no conflict of interest.

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