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Original Article

MOLECULAR DOCKING STUDY AS THERAPEUTIC APPROACH FOR TARGETING CHOLECYSTOKININ IN PANCREATIC CANCER

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ABSTRACT

Objective: The Cholecystokinin A receptor (CCK-ARs), also known as CCK1 receptor, is a type of G protein-coupled receptor that is primarily responsive to the hormone Cholecystokinin (CCK). CCK-ARs is one of the receptors characterized and validated to promote pancreatic cancer progression. Devazepide is a selective antagonist of the CCK-ARs. This study aims to find a potential ligand that has the most effective and representative interaction with cancer receptors, becoming a new therapeutic effect using molecular docking Molecular Operating Environment (MOE) with receptor code 7F8U.

Methods: We conducted an in silico study by docking candidate ligands with Cholecystokinin Receptor (CCKRs) using the MOE 2015 V.10 application. The ligands of choice come from natural ingredients such as curcumin, resveratrol, berberine, baicalein, dioscin, wogonin, and piperine. Validate the receptor with the Root mean Square Deviation (RMSD) value and docking results with the GIBBS S value.

Results: 6 compounds, such as curcumin, resveratrol, berberine, baicalein, wogonin, and piperine, were selected for docking as candidates to determine whether they have interactions with CCK-ARs. Based on the docking results, the Gibbs values obtained were -14.9522;-12.4566;-15.5033;-12.6961;-13.4234;-11,6130 joules/kg. mol, berberine is the compound with the lowest Gibbs energy, namely -15.5033 joules/kg. mol and is one of the strongest. The interactions that occur include Methionine A121-side chain donor, Methionine B121-side chain donor, asparagine A333-amine group and nitrogen atom, B333-amine group and nitrogen atom, Arginine A336-negative oxygen atom, and B336-negative oxygen atom.

Conclusion: Berberine which is a natural alkaloid, is suitable for devazepide, which is a positive control for ligand interactions when tethered to the CCKRs. This finding could be a potential new drug for pancreatic cancer. However, further studies, such as *in vitro*, *in vivo*, and clinical trials need to be conducted for ordering activity, safety, and safety of new drugs.

Keywords: Pancreatic cancer, CCK, Molecular docking, In silico, 7F8U, Barberine

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INTRODUCTION

One of the deadliest cancers in the world is pancreatic cancer. This cancer has increased in cases in the last 2 decades with very high morbidity and mortality rates. Smoking, a family history of chronic pancreatitis, advanced age, being a man, having diabetes mellitus, being overweight, and having helicobacter infection are all risk factors for this cancerous condition [1].

In the military world, pancreatic cancer has the greatest influence as a deadly disease for military personnel. Military personnel areat very high risk of developing pancreatic cancer up to 75% annually [2]. As well as something invisible the military's susceptibility to pancreatic cancer after occupational exposure to chemical carcinogens and tobacco use. In addition, exposure to tobacco due to smoking habits is very high among military personnel. Cigarette smoke, such as Polycyclic Aromatic Hydrocarbons (PAHs) and heavy metals, contribute to pancreatic cancer rates in military personnel [3].

Surgical resection is currently the most well-known and successful treatment for pancreatic cancer, even though it still frequently manifests later than expected. Therefore, currently, the latest treatment derived from natural ingredients for cancer is being developed because it is effective and lacks serious side effects. Through experimental research, the anticancer properties of natural products were examined in cases of lung, breast, colon, pancreatic, and prostate cancer. Curcumin, resveratrol, berberine, baicalein, dioscin, wogonin, piperine, and other natural substances are among those said to have anti-cancer potential. Additionally, it is established that this natural substance causes more cancer cells to undergo apoptosis than healthy cells do. Consequently, natural products will be crucial in developing innovative cancer therapies in the future [4].

Cholecystokinin (CCK) is one of the receptors characterized and validated to promote the development of pancreatic cancer. Pancreatic cancer cells, fibroblasts, and lymphocytes contain Cholecystokinin Receptor (CCKRs) [5]. In humans, pancreatic cancer has Subtypes of Cholecystokinin A receptor (CCK-ARs) and CCK-BRs, with one of the inhibitors associated with CCK-ARs being devazepide. Devazepide is an exceptionally potent and selective CCK-ARs antagonist [6]. CCK antagonists have been investigated as growth inhibitors in particular cancers [7]. Devazepide is the most powerful and selective CCKRs antagonist, with about 160-fold more affinity compared to the CCK-BRs, for the CCK-ARs [8].

The method used is molecular docking, which aims to find suitable ligands or drug candidates for CCK targets. The in silico structure-based technique known as molecular docking enables the discovery of novel medicinal compounds, the prediction of molecular interactions between ligands and targets, and the characterization of Structure-Activity Relationships (SARs) without previous knowledge of the chemical structure of other potential target modulators [9]. Molecular docking provides the benefit of allowing us to see the interactions between ligands. This research intends to identify candidates for multiple ligands that interact with the CCKRs in pancreatic cancer and have more activity than devazepide, having the potential to be created as innovative treatments for pancreatic cancer.

MATERIALS AND METHODS

Material

The receptor Data were downloaded from http://www.rcsb.org's Protein Data Bank (PDB). Table 1 describes selected ligands generated from natural substances, including curcumin, resveratrol, berberine, baicalein, dioscin, wogonin, and piperine. Devazepide served as our positive control (fig. 1). The 3D structure of the chemical was retrieved from the PubChem database in SMILES format.

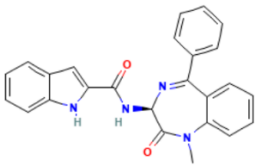
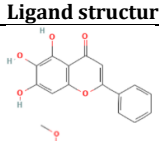
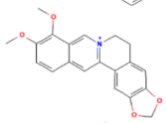
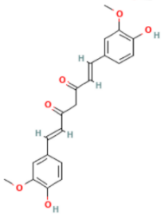
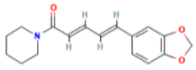
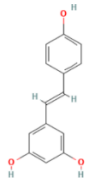
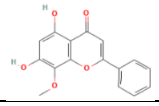


Fig. 1: Molecular structure of devazepide as the positive control

Table 1: Plant and ligand structures used for this study

Plant	Ligand	Ligand structure	IUPAC name
Scutellariabaicalensis	Baicalein		5,6,7-trihydroxy-2-phenylchromen-4-one
Berberis vulgaris	Berberine		16,17-dimethoxy-5,7-dioxa-13-azoniapentacyclo[11.8.0.0[2,10].0[4,8].0[15,20]]henicosa-1(13),2,4(8),9,14,16,18,20-octaene
Curcuma longa	Curcumin		(1*E*,6*E*)-1,7-bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione
Piper nigrum	Piperine		(2*E*,4*E*)-5-(1,3-benzodioxol-5-yl)-1-piperidin-1-ylpenta-2,4-dien-1-one
Vitis vinifera	Resveratrol		5-[(*E*)-2-(4-hydroxyphenyl)ethenyl]benzene-1,3-diol
Tetracera indica	Wogonin		5,7-dihydroxy-8-methoxy-2-phenylchromen-4-one

Methods

Ligand and receptor docking

CCKRs determination in pancreatic cancer

Receptors and existing drugs are searched as positive controls associated with pancreatic cancer from Pubmed. The CCK PDB, RCSB was consulted for the receptor file with the identification code 7F8U. MOE tool is used to examine the structure of the receptor file with the code 7F8U. mbd extension [10, 11].

Preparation of receptors with validation

Protonation at the 7F8U receptor by adding a proton in the form of a hydrogen cation (H+ion) to the molecule to determine the gasteiger charge and polar bond for each atom in the molecule, as well as to correct the partial charge estimate. MOE conducted validation of the docking approach utilizing two determining the target protein's and its original ligand's RMSD value was used to validate the docking approach. RMSD is a measure used to determine the similarity between flexible and stiff crystallographic result interaction techniques and docking ligands [12]. The target protein is considered legitimate if the RMSD value is less than 2 [13].

Docking of the molecules to the receptors

Using the MOE software, parameters and data are acquired to establish the optimal ligand-receptor interaction, which is then contrasted with positive controls and valid receptors with ligands [10, 11].

Analysis and visualization of docking results

Determination of the conformation of the docking ligand (best pose) is done by choosing conformational ligands that have bonded energies the lowest. Docking results with pose best then analyzed using Discovery Studio. Analyzed parameters include amino acid residues, hydrogen bonds, predictive inhibition constant, and free energy bonds. Determination based on bond-free energy is indicated by the docking result which has the most negative (S) value [10, 11].

In sillico toxicity prediction

The toxicity prediction process begins with searching for the structure of the compound to be tested by entering the canonical SMILE structure using the PubChem website (https://pubchem.ncbi.nlm.nih.gov/). ProTox II website (Sttps://tox new.charite.de/protox_II/index.php?site=compound_input), Enter the canonical SMILE structure, select additional models for prediction including organ toxicity, endpoint toxicity, Tox21 Nuclear receptor signaling pathways, Tox21 Stress response pathways. The results are obtained in the form of LD50 levels and categorized into 5 types of LD50 levels.

RESULTS AND DISCUSSION

We performed ligand validation and docking procedures on several ligands, obtained the S value (Joule/kg. mol), and described the relationship between the ligand and the receptor. The free bond

energy value is determined as follows, it is found that the largest S value (Joule/kg. mole) is found in the natural compound berberine with an S value of -15.5033, which has the following bond description.

Table 2: The result of CCK (7F8U) docking of the receptor with several ligands

Ligand	ΔS (Joules/kg. mole)	Ligand and receptor interaction
Baicalein	-12,6961	→ Cysteine 195
Berberine	-15,5033	NH_3 → Asparagin A333 and Asparagin B333 N → Asparagin A333 and Asparagin B333 O^- → Arginin A336 and Arginin B336 Sidechain → Metionin A121 and Metionin B121
Curcumin	-14,9522	There is only ligand exposure and receptor exposure
Piperine	-11,6130	R=O → Phenilalanin 198 Sidechai → Cysteine 94
Resveratrol	-12,4566	R-OH → Asparagin 98 → Histidin 210 R-OH → Histidin 210
Wogonin	-13,4234	→ Asparagin 98

The following is an illustration of the interaction between potential ligands from natural products and the CCKRs (7F8U). It was found that the natural product berberine ligand had the most bonding interactions between the ligand and the receptor and the ΔS value was very dominant.

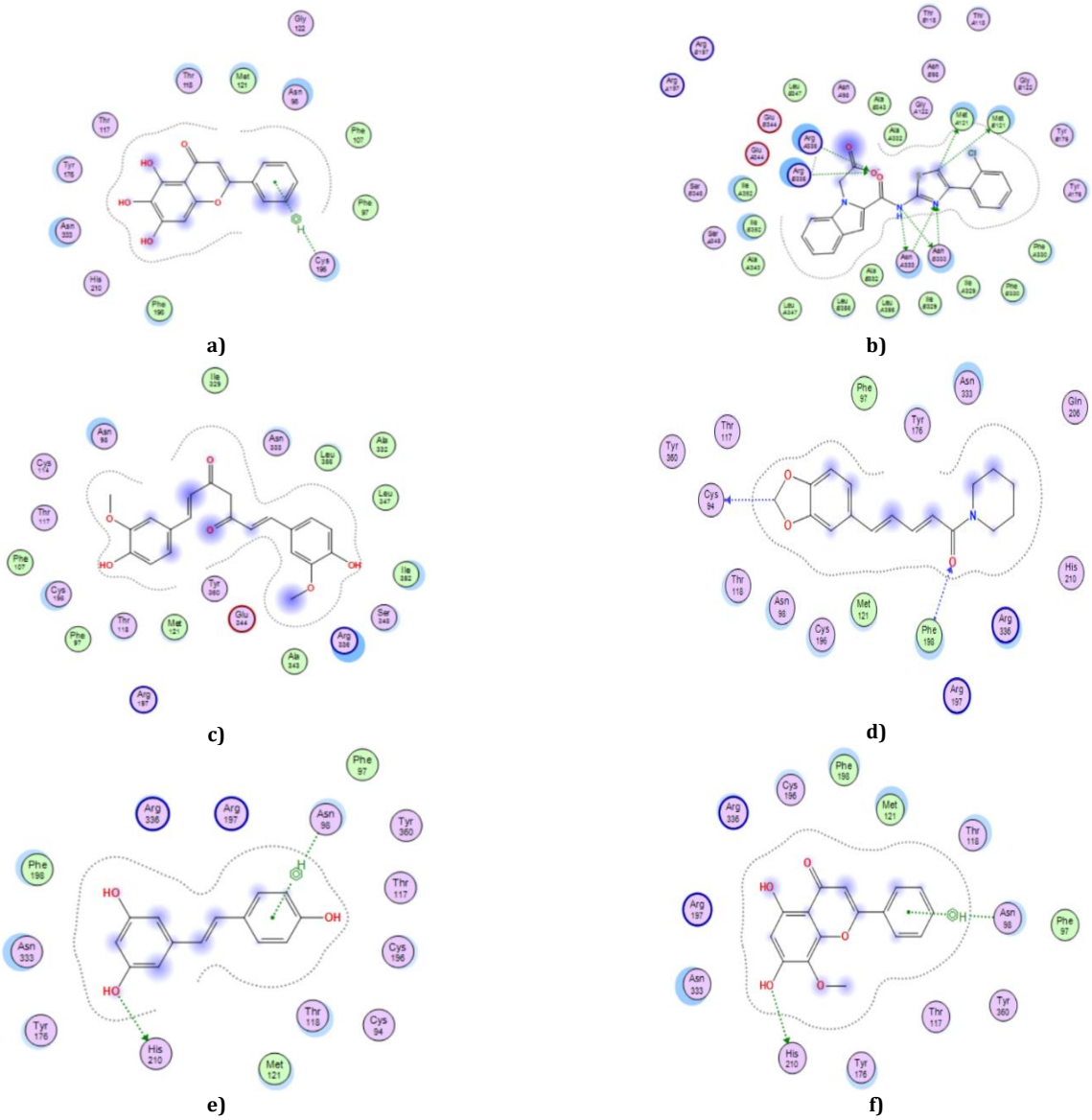


Fig. 2: Ligand interaction with CCKRs (7F8U), a). Baicalein, b). Berberine, c). Curcumin, d). Piperine, e). Resveratrol, f). Wogonin

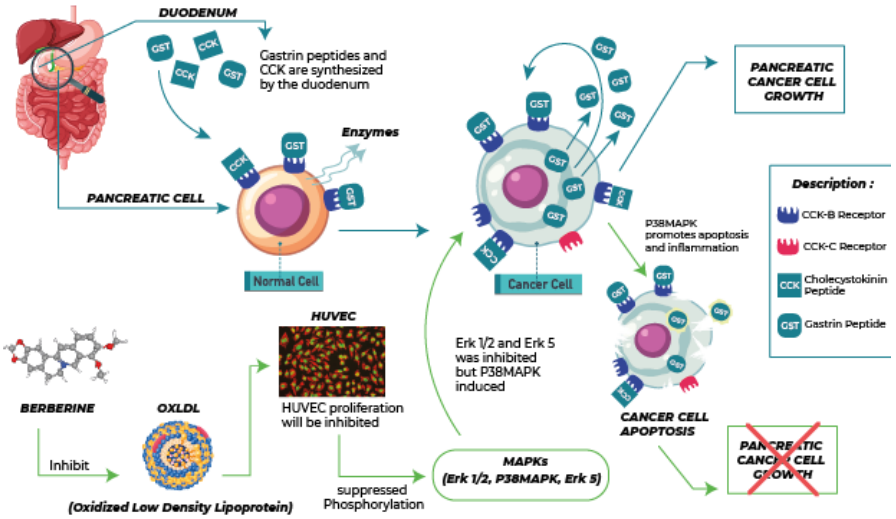


Fig. 3: Formation of pancreatic cancer cells mediated by exogenous peptide gastrin and CCK at the CCK-BRs. The alkaloid berberine is an isoquinoline that has therapeutic potential. Berberine offers pharmaceutical uses such as those for treating cancer, oxidase, hyperglycemia, and Low-Density Lipoprotein production (LDL). Berberine dramatically slows the growth of HUVEC that is induced by LDL. As is known, oxLDL increases HUVEC proliferation by producing O2 through the NAD (P) H oxidase. The result of the oxLDL-induced proliferative HEVECs is the inhibition of MAPKs via phosphorylation. MAPKs activate the ERK 1/2, p38MAPK, and ERK 5 signaling pathways. From the situation when ERK 1/2 and ERK 5's functions as growth, differentiation, and development will be hampered. In contrast, p38MAPK will be activated to initiate the processes of inflammation, apoptosis, cell proliferation, and differentiation. The MAPK p38MAPK inhibitor will increase apoptosis and inflammation in the pancreatic cancer subtype and inhibit gastrin peptide CCK-BRs. Cell is produced normally since the cancer cell has already degraded.

CCKRs is one of the receptors found and defined in pancreatic cancer; this receptor is overexpressed in pancreatic cancer. Signaling in pancreatic cancer is started when the CCKRs ligand gastrin or CCK is activated, which results in cell proliferation. In human pancreatic cancer, gastrin is overexpressed and has been discovered to accelerate growth through an autocrine mechanism. It is well known that in this case, pancreatic carcinogenesis and cancer development are primarily fueled by the CCK-BRs pathway. Gastrin and CCK peptides are generally synthesized in the duodenum and not in the pancreas [14].

CCK-A, which predominates in the normal murine pancreas, and CCK-B, which predominates in the normal human pancreatic, have been identified as the two kinds of CCKR. In normal human pancreatic tissue, the CCK-BRs phenotype serves as the primary receptor. Exogenous peptide gastrin and duodenal CCK go via the peripheral circulation and connect to the CCK-BRs to initiate intracellular signaling and release digesting pancreatic enzymes in the typical human pancreas. The CCK-BRs is overexpressed in pancreatic cancer, and cancer cells produce the peptide gastrin as a result. These circumstances drive cancer cells to secrete Gastrin-17, which binds to additional CCK-BRs and stimulates cell proliferation through an autocrine process [15].

Berberine is a naturally occurring isoquinoline alkaloid identified from *Coptis chinensis*, *Berberis aquifolium*, and *Berberis aristate*, among other therapeutic plants. In recent studies, berberine offers anti-tumor, anti-oxidation, anti-hyperglycemic, and LDL-lowering characteristics as well as other pharmaceutical purposes. In cancer, one of the key steps is to inhibit cell proliferation. Research has demonstrated that the alkaloid compound berberine, when used as a chemopreventive medication, can effectively target several tumors by altering various signaling pathways. Because of their restricted water solubility, Numerous nanotechnological methods have been developed to aid in their dispersion across cell membranes. Two of the six clinical trials under investigation are already complete [16]. By inhibiting the expression of PCNA, NF-kB, and LOX-1, Berberine dramatically inhibits the proliferation of oxLDL-induced HUVECs. As is well known, oxLDL enhances HUVEC proliferation via NAD(P)H oxidase-derived O2 production. The result of oxLDL-induced inhibition of HEVECs proliferation is suppression of MAPKs phosphorylation. MAPKs produce signals in the form of ERK 1/2, p38MAPK, and ERK 5. Of the three forms of signaling, conditions are generated where the work of ERK ½ and ERK 5 will be inhibited from carrying out their functions as growth, differentiation, and development. Meanwhile, p38MAPK will be induced to carry out its function in the form of inflammatory processes, apoptosis, growth, and differentiation. Ultimately, signaling MAPKs p38MAPK will induce apoptosis and inflammation in pancreatic cancer cells and inhibit gastrin peptides against CCK-BRs. Normal cells are produced because cancer cells have been degraded [17].

7F8U was selected to be the receptor used for docking in this study which was taken from the RCSB PDB. CCKARs–lintitript, CCKARs–devazepide, and CCKARs–NN9056 structures' atomic coordinates have been submitted to the PDB RCSB with the corresponding accession numbers 7F8U, 7F8Y, and 7F8X, respectively [18]. CCKRs selection is based on the percentage value of positive tumors in pancreatic cancer of 67 to 100; CCKRs-positive tumors are often characterized by neuroendocrine differentiation, as assessed by immunohistochemical synaptophysin [19]. Devazepide (L-364.718) was used as a positive control which was used as a comparison to determine the active constituent (ligand). The selective CCKRs antagonist devazepide (L-364.718) suppresses the growth of human pancreatic cancer cell lines, according to research [20].

Preparation and validation of 7F8U

Preparation of 7F8U protein by separating the protein from the native ligand so that there is space (pocket/cavity) that will be used during the berberine docking process on the protein. The result of this protein preparation process is a protein without the original ligand and the original ligand which is saved in the form of a pdb file.

Docking the 7F8U back via a 3D protonation procedure utilizing the moe program served to validate the molecular docking approach. The validation parameter of the method used is the RMSD value. It compares the atomic locations between the experimental structure and the structure docked to the protein to measure two postures. The method is considered valid if the resulting RMSD 2 value is valid [22]. The lesser the RMSD number, the higher the quality of predicted ligand conformation will be since it will be closer to the original conformation [23].

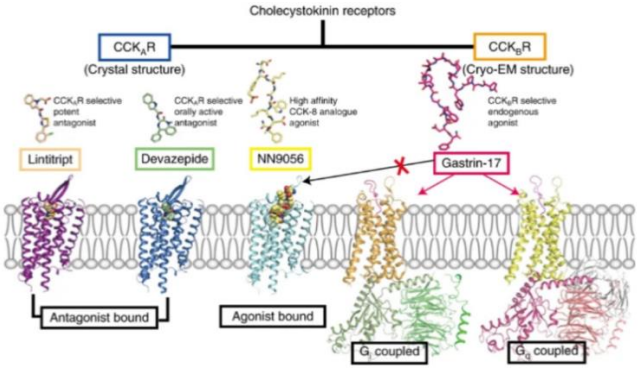


Fig. 4: Agonist and antagonist inhibitor for CCKRs. The neurointestinal peptide hormone receptors known as CCKARs and CCKBRs for CCKplay a significant role in the control of appetite and food intake. Here, we provide two CCK-BRs-gastrin cryo-electron microscopy structures in complex with Gi2 and Gq, as well as three crystal structures of human CCKARs in complex with various ligands, including one peptide agonist and two small molecule antagonists. The molecular underpinnings of peptide selectivity in the CCKRs family are revealed by this structure, along with the pattern recognition of various types of ligands. Our findings offer atomic insights for various receptor activation and ligand recognition pathways when combined with pharmacological information. These understandings will aid in the identification of possible treatments that target the CCKRs [21]

Docking of several compounds with CCKRs (7F8U)

Docking ligands on the active site of the CCK 7F8U receptor using the Moe 2015.10 program with induce fit refinement settings with a minimum of 20 poses. In the form of the bond energy value between the receptor and ligand, the docking procedure predicts the interaction activity between the ligand and receptor. According to the Gibbs energy hypothesis, the binding between a ligand and its receptor is more stable, the less energy is created during the bonding process. The bond energy shows the affinity between cyanidin and peonidin with the protein; the lower the bond energy obtained, the more stable the bond formed [24].

The outcome of the docking method is a prediction of the interaction activity between the ligand and the receptor. The process of molecular docking facilitates the determination of the binding geometry between two molecules that interact and have known structures. The process of docking foretells the ideal alignment of the ligand and receptor to form a stable complex [25]. Six compounds were chosen as candidates for docking to evaluate if they interact with theCCKRs. Based on the outcomes of the docking, the free energy values of 8 compounds, baicalein, berberine, curcumin, piperine, resveratrol, and wogonin were-12.6961, respectively;-15.5033;-14.9552;-11.6140;-12.4566;-13.4234. This value is formed due to several interactions with receptors such as side chain donors for Methionine A121 and B121, asparagine A333 and B333 with amine groups and nitrogen atoms, Arginine A336 and B336 with negative oxygen atoms, cysteine 195 with benzene, and so on. However, of the 6 compounds, some compounds do not have direct interactions, such as curcumin. Curcumin has only ligand-exposed and receptor-exposed areas. This implies that the ligand does not interact with the protein's active site [26].

Of the 6 ligands that were successfully attached to the 7F8U receptor, the ligand compound with the strongest interaction was berberine because it had a free energy Gibbs S value of -15.5033 Joule/kg. Mole. Occurs due to several interactions with receptors such as side chain donors for Methionine A121 and B121, asparagine A333 and B333 with amine groups and nitrogen atoms, and Arginine A336 and B336 with negative oxygen atoms. One of the berberine factors has the highest S value due to the presence of hydrogen bonds. The strongest dipole-dipole force occurs in hydrogen bonds, which are the connections that connect hydrogen atoms in one molecule to other elements (N, O, and F) in other molecules [27]. In addition, according to Prananto, hydrogen bonds are electrostatic interactions between atoms that are hydrogen bonded to each other's electronegative atoms [28]. Meanwhile, Kurniawan and Nur claim that protons' dynamic movements inside a bond are properties of hydrogen bonds' constituent protons [29]. These molecular interactions with one another, which produce hydrogen bonds, typically take the appearance of hard-to-see dashes. The creation of newly established bonds hydrophobic interaction, the Van Der Waals forces, and the amount of hydrogen in the container all have an impact on energy [30, 31]. The more complex the compounds with interacting proteins are, the lower the bond energy formed and the stronger the bond between the two [31-36]. The Gibbs S free energy value of berberine compounds is more negative than that of devazepide. The more negative the Gibbs energy value, the stronger hydrogen bond [31-37]. Therefore, the berberine compound became a comparable compound with positive control among all docking ligands. Berberine is the primary isoquinoline alkaloid isolated from the traditional Coptis plant, and it has a variety of pharmacological properties, including antimicrobial, antidiabetic, and anti-inflammatory. Berberine has a positive therapeutic impact on cancer by adjusting the apoptotic and signal transduction pathways' activity, eventually slowing the proliferation of malignant cells. Moreover, A topoisomerase II (topology II) inhibitor called berberine may prevent the production of topoisomerase-mediated Splicing complexes for DNA, hence influencing the DNA autonomic replication process to trigger tumor cell death [38].

Toxicity prediction of berberine compound

The scoring was to identify potential lead compounds. The binding affinities were compared with known anticancer drugs, devazepide. The interaction analysis between ligand and protein active site residue identified vital interactions. The structure-activity relationship was done by comparing the compound's chemical structure with predicted activity. Virtual ADME analysis was done using ADME software, and the values were compared [39]. Promising binding energy to the target receptor is not the only criterion for promising drug candidates. The secret to success in drug development is comprehending a drug candidate's entire mechanism of action within the human body. Furthermore, toxicity analysis of possible drug candidates is crucial for the preliminary assessment of a medicine's potential hazard to humans. Researchers can screen medication candidates more quickly and effectively by using a computer to analyze toxicity, which reduces the time and expense associated with the research and development phases. Additionally, this information may steer the development of safer and more effective drug candidates and assist prevent clinical trial failures. The overall results of the toxicity analysis show that berberine is very safe to use orally and in inhalation. However, you need to be careful about using berberine directly on the skin, especially for people who have sensitive skin, because it might cause an allergic reaction. Apart from that, the Bolied-egg diagram shows that berberine can penetrate well into the BBB as indicated by the location of berberine in the yellow area. From these results, berberine has the potential to be a safe and effective drug candidate in all drug administration routes that works as a trigger for apoptosis and cell death through the CCKRs which can be useful for pancreatic cancer sufferers.

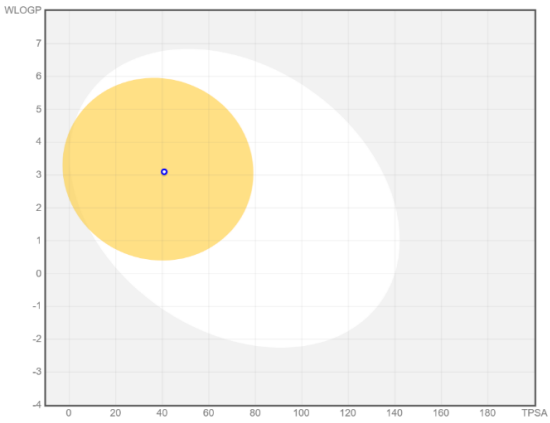


Fig. 5: Penetration ability in the Blood Brain Barrier (BBB) and toxicity value of berberine

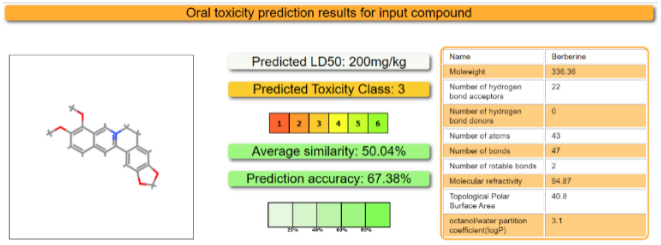


Fig. 6: In sillico toxicity prediction with ProTox II website

Based on the type of compound, method of administration, and type of organism, the LD50 value of berberine varies greatly. In the predicted results of the oral toxicity test using the ProTox-II application, the predicted LD50 of the berberine compound was 200 mg/kg. These results show that the predicted level of toxicity of the berberine compound in the predicted oral toxicity test is at level 3, where the conclusion is that this berberine compound is likely to be of the moderately hazardous type (50<LD50 ≤ 300).

This is also explained further in the following table.

Table 3: Globally harmonized system of classification and labelling of chemicals (GHS) and WHO guidelines of chemical hazardous

LD_{50} (mg/kg)	≤ 5	5-50	50-300	300-2000	2000-5000	> 5000
GHS	Category 1	Category 2	Category 3	Category 4	Category 5	Category 6
WHO guidelines	Ia (extremely hazardous)	Ib (Highly hazardous)	II (moderately hazardous)		III (Slightly hazardous)	IV (Non-hazardous)

Chemical hazards are divided into four classifications according to the WHO's 1978 classification guidelines: Class Ia (very hazardous), Class Ib (very hazardous), Class II (moderately hazardous), Class III (somewhat hazardous), and Class IV (non-hazardous). These guidelines have periodically been updated and republished. International criteria for chemical categorization and labeling are provided by the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO). Significant national roles are played by these voluntary recommendations, particularly in developing nations. For the GHS to be implemented, the agricultural sector is essential, and both organizations are thinking about aligning the classification of industrial chemicals with pesticides. The FAO Guidelines must be updated before the WHO Guidelines, nevertheless. GHS was included in the 2009 WHO Guidelines update. The revised recommendations included both acute and chronic toxicity, particularly for active components, and eliminated the distinction between classification criteria for liquids and solids. They also kept the previous WHO Classes. For instance, the WHO categorized captafol as extremely dangerous due to its carcinogenicity in rats and mice, despite the drug being classed as GHS Category 5 for acute oral toxicity [40].

Under the GHS, the toxicologist (or ecotoxicologist) will continue to play a crucial role in the current chemical hazard classification systems. Toxicologists are in charge of creating the data (*in vitro* tests, ecotoxicological and toxicological research) that will be utilized in the classification process. They are also crucial in the interpretation of that data. When the data meets the numerical criteria specified in the GHS, categorization may be necessary for specific hazard endpoints, and it won't take much expertise to determine that classification. For instance, a chemical that causes acute toxicity in rats and has an approximate oral LD50 of 200 mg/kg will be classified as category 3. Substances and mixtures are categorized according to the entire "weight of evidence," which takes into account data from animal experiments, human experience, and *in vitro* testing. This holds for all danger classifications, including irritancy and skin corrosivity. For example, data from animal research, human studies, in vitro testing, and pH information can all be used to determine the proper classification. Using this weight of evidence method, the toxicologist's judgment is needed to decide the proper classification. Since single animal studies frequently yield insufficient data, the weight of evidence technique is essential for classifying chemicals for carcinogenicity, germ cell mutagenicity, or reproductive toxicity. Studies on the site and manner of action are combined with data from *in vitro* experiments, animal experiments, and human experience. The key is expert toxicological judgment. Because of worries about animal cruelty, the use of validated *in vitro* tests in hazard classification is expanding. To classify compounds or combinations using the weight of evidence methodology, a data package is utilized to assess the robustness of the methodologies that toxicologists have developed and researched [41].

Cardiotoxicity, cytotoxicity, genotoxicity, mutagenicity and carcinogenicity

Berberine appears to have several pharmacological properties, including immunomodulatory, antioxidative, cardioprotective,

hepatoprotective, and renoprotective actions, according to numerous clinical and experimental research [42]. In addition, it has been proven that berberine has an inhibitory effect on the potassium rectifier current so that it delays and the hERG coating increases the action potential, which can have antiarrhythmic and arrhythmogenic properties. Berberine vulgaris showed a positive impact on heart contractility, blood pressure reduction, and protection against reperfusion injury [43]. Several studies have also reported that berberine is a good antitumor in both *in vitro* and *in vitro* tests; therefore, it is called a valuable anticancer drug. Berberine has a certain level of toxicity in normal cells, which has the basic mechanism of inhibiting adenine nucleotide translocase which is followed by a decrease in energy production. One of them is berberine in 10 mmol, which can induce the most toxic potential after 2 h of treatment; this compound increases oxidative stress and reduces nerve viability [44].

It has been demonstrated that berberine benefits both healthy and malignant cells *in vivo*. Berberine was discovered to decrease tumor multiplicity in a mouse model of colorectal carcinogenesis in a study conducted in 2015 by Li and colleagues. In a different study, mice were given injections of SiHs cells to assess the impact on tumor growth and lung metastasis. Treatment with berberine reduced lung metastasis, pulmonary weight, tumor size, and angiogenesis. It exhibited mild inhibitory effects on tumor development and weight in a 4T1 breast cancer model in mice. Anti-DR5 and berberine together enhanced their antitumor effects and decreased the occurrence of lung metastases. Additionally, berberine therapy reduced the size and growth of tumors created by implanting S 180 sarcoma tumor cells into Kunming mice as well as prostate cancer PC-3 and LNCaP cells into BALB/c athymic mice. Goldenseal contains berberine, which has been shown to cause double-strand breaks in cells lacking Rev3. The comet assay test demonstrates that via blocking topoisomerase 1, raising histone H2A. X phosphorylation, inducing cell cycle arrest, and activating checkpoint-related proteins, berberine, and goldenseal causes DNA damage in HeLa and HepG2 cells. In haploid yeast cells grown in non-growth circumstances, berberine chloride is nonmutagenic; yet, in dividing cells, it increases the frequency of cytoplasmic PETITE mutations and HOM3 frameshift reverts. One could classify berberine's phototoxicity as genotoxicity. Berberine causes photodamage to the eyes and increases the risk of illnesses affecting the lens epithelial cells when it is used in eye drops and lotions. When human HaCaT keratinocytes are exposed to UVA radiation at a concentration of 50 μM, berberine results in an 80% reduction in cell viability and single-strand breaks in DNA. It's noteworthy to note that berberine's phototoxicity depends on its solvent. In nonpolar solvents like CH_2Cl_2, berberine radiation produces both O2 and radical species, but not in aqueous solutions. It is important to take UVA protection into account when using berberine topical treatment [43, 45].

Table 4: A collection of previous *in vitro* studies that tested the berberine compound on the target of several cancer cell lines so that it can become a reference for further studies

Model	Treatment berberine	Effects to cell	Mechanism action	Reference
BxPC-3	10–200 μM berberine 24–72 h	↓ Proliferation	↑ Caspase 3/7	[46]
PANC-1	0.3–6 μM berberine 17–72 h	↓ Proliferation	↑ pAMPK (Thr172)	[47]
MiaPaCa-2		↑G1-phase population	↑ pACC (Ser79)	
		↓S and G2/M-phase population	↓ mTORC1	
		↓Mitochondrial membrane potential	↓pp70 S6K (Thr389)	
		↓ ATP levels	↓ pS6 (Ser240/244)	
			↓pERK (Thr202/Tyr204)	
			↑ pRaptor (Ser792)	
Mia-PaCa-2	15 μM berberine 72 h	↓ CSC population	↓ SOX2	[48]
PANC-1			↓ OCT4	
			↓ NANOG	
PANC-1 Mia-PaCa-2	1–15 μM berberine 72 h	↑G1-phase population	↑ Caspase-3/7 activity	[49]
		↓S-phase population		
		↑ Apoptosis		
		↑ ROS		
Mia-PaCa-2	10–50 μM berberine 1–48 h	↓ Citrate synthase activity	↑ p21	[50]
		↑G1-phase population	↑ Caspase-3 activity	
		↓ S and G2-phase population	↑ LC3	
		↑ Senescence	↑ DAP1	
		↓ Migration	↓ CXCR4	
		↓ Invasion	↑DNMT1	
			↑DNMT3A	
			↑ DNMT3B	
			↑ MGMT	
Panc-1	1–60 μM berberine 48–72 h	↑ Apoptosis	↓ TNFα	[51]
		↓ Metastasis	↓ CA242	
		↑Glycolysis-associated metabolites	↓ K-Ras	
		↑Glutamine-associated metabolites	↑ CDKN2A	
		↓ Citric acid cycle-associated metabolites		
		↑Mitochondrial damage		
		↓Citrate metabolism		
PANC-1 AsPC-1 SW1990	0–30 μM berberine 24 h	↓ Trans-endothelial migration	↓ pSmad2	[52]
			↓ pSmad3	
			↓ SNAIL1	
			↓ SLUG	
TGF-βtreated Primary acinar cells	ADM induction: 5 ng/ml TGF-β 2 Days 10 μM berberine 1–2 d	↓ ADM	↓ CK19	[53]
		↓ Glycolysis	↓ LDHA	
			↓ALDOA	
			↓ PFKL	
			↓ PKM2	
			↓ PDK1	
			↑ pAMPK	
			↓ pmTOR	
			↓ HIF-1α	
MIN6	2.5–50 μM berberine 2–24 h	↑ Apoptosis	↑ Cytochrome C	[54]
		↑DNA fragmentation	↑ AIF	
			↑ Apaf-1	
			↑ Bax	
			↑ Cleaved Caspsase-3 ↑ Cleaved PARP	
			↓ Bcl-2	

Literature study of *in vitro* analysis cells cultured of pancreatic cancer

High concentrations of berberine (10–200 μM) have been shown to inhibit the growth of pancreatic cancer cells and induce caspase-independent cell death in BxPC-3 human pancreatic adenocarcinoma cells and HPDE-E6E7c7 normal human pancreatic ductal epithelial cells. Berberine treatment significantly increased caspase-3 and-7 activity in both cell lines, suggesting that berberine induces apoptosis in cancer cells at high concentrations. Additionally, berberine induces apoptosis by mechanisms that do not require caspase. Berberine or lovastatin therapy decreased cell viability in a dose-dependent manner, and the combination of the two drugs had extremely advantageous cytotoxic and cytostatic effects. Pre-treatment with products of the mevalonic acid pathway decreased the anticancer effects of lovastatin but not berberine, indicating that berberine boosts the anticancer effects of lovastatin independently of the cholesterol synthesis process. After being exposed to berberine for 17–72 h, pancreatic cancer cells (PANC-1 and MiaPaCa-2) had reduced mitochondrial membrane potential and ATP levels, an increase in the number of cells in the G1 phase, and inhibition of DNA synthesis and proliferation. This was similar to the well-known inhibitor of mitochondrial complex I, metformin, which inhibits the growth of tumors. Moreover, berberine suppressed the activation of ERK and mTORC1 induced by insulin and neurotensin in a concentration-dependent manner. Furthermore, berberine decreased the proportion of side population (SP) cells in PANC-1 cells, downregulating genes associated with stem cells. After being exposed to 1–15 μM berberine for 72 h, PANC-1 and Mia-PaCa-2 pancreatic cancer cells showed increased G1 phase population, concentration-dependent reduction of cell growth, and activation of apoptosis. The notion that berberine's effects are ROS-dependent is suggested by the concentration-dependent increase in intracellular ROS levels that accompanied the compound's proapoptotic impact.

Berberine, a material found in the cytoplasm of MiaPaCa-2 cells, has been shown to suppress mitochondrial function and induce autophagy. Berberine treatment significantly decreased cell migration and invasion and enhanced the mRNA expression of DNA methyltransferases (DNMT) in wound-healing experiments. Furthermore, berberinetherapy inhibited the growth of pancreatic cancer cells by lowering the expression of tumor necrosis factor α, carbohydrate antigen 242, and K-Ras, three oncogenic proteins. berberine elevated metabolites associated with energy metabolism and decreased metabolites associated with the citric acid cycle because it damaged mitochondria. Berberine therapy suppressed the viability of AsPC-1, PANC-28, and MIA-PaCa-2 pancreatic cancer cells and decreased their colony formation in a concentration-dependent manner. In MIA-PaCa-2 cells, a TP53 gain-of-function mutation results in the synthesis of a p53 protein with a modified DNA binding motif. It has been demonstrated that berberine has anticancer capabilities that could prevent the spread of pancreatic cancer cells by fortifying the pulmonary vascular barrier. By binding to TGFBR1, berberine inhibits its kinase activity, protecting the endothelium barrier. In one study, berberine decreased CK19 levels, increased amylase levels, and decreased the induction of ADM in cells treated with TGF-β. Furthermore, berberine attenuated the Warburg effect, permitting lactate production and glucose consumption to resume in acinar cells treated with TGF-β. berberine also increased activated levels of AMPK and decreased active mTOR and HIF-1α. Compound C, an AMPK inhibitor, restored glycolysis and prevented berberine from inhibiting PanIN development. After berberine treatment, the number of apoptotic cells and pro-apoptotic chemicals increased, and concentration-dependent reductions in cell viability were seen in MIN6 insulinoma cells.

CONCLUSION

Berberine, which is a natural alkaloid, is suitable for devazepide, which is a positive control for ligand interactions when tethered to the CCKRs. This finding could be a potential new drug for pancreatic cancer. However, further studies, such as *in vitro*, *in vivo*, and clinical trials need to be conducted for ordering activity, safety, and safety of new drugs.

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AUTHORS CONTRIBUTIONS

OA: Protein Preparation, Docking, Molecular Modelling, TAR: Analysis Data, Developed the Concept and Design Manuscript, ER: Helped Revised Manuscript

CONFLICT OF INTERESTS

There is no conflict of interest in this research

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