In Silico Inhibitor Analysis of Ran Binding Protein 9 (RanBPM) in Non-small Cell Lung Cancer (NSCLC)

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Non-small cell lung cancer (NSCLC) is the overexpression of RanBPM, which causes an imbalance in the protein, which can affect the cancer progression. RanBPM is a protein found in the nucleus and cytoplasm of the cell, which is necessary for multiple different cell functions. RANBP9s imbalance can cause increased cancer progression. We hypothesize that the ligands bind to the RanBPM protein and could inhibit the RanBPM protein functioning, thus preventing lung cancer. In the current research work, we have performed computational simulations so the RANBP9 protein can be inhibited, which can hinder the NSCLC. To perform this simulation, we first need RanBP9 and chemical ligands. The Protein Data Bank (PDB) was used to get the 3D structure of the RanBP9 protein. The software AutoDock vina was used for the molecular docking simulations. The druggable site and predicted by the P2rank (predicted by machine learning) and that molecular docked structures were identical that further validating the molecular docking results. We used PLIP analysis to predict the interaction between the ligands and RANBP9. To understand the pharmaceutical properties of the ligands, we have used the SwissADME web server, which showed that the ligands have high GI absorption and a high blood-brain barrier, meaning that these drugs can easily be absorbed from the stomach and taken orally. This research paves the way for precision therapies targeting RanBP9 in NSCLC, enabling novel drug development and biomarker-based diagnostics.

Keywords: Non-small cell lung cancer, ran binding protein 9 (RanBPM), Molecular Docking Simulations, Computer Aided Drug Design.

Introduction

Ran-binding protein 9 or RANBP9 is a scaffold protein necessary for homeostasis and various cell functions[1]. The RANBP9 gene is present in many body parts, including tissues, parts of the cell, and organs like the heart. RANBP9 is involved in nucleocytoplasmic transport[1]. This process occurs in eukaryotic cells, and it is when molecules move through nuclear pore complexes to move between the nucleus and cytoplasm[2]. RANBP9 is a binding partner of the small GTPase Ran, and it takes part in the management of the transport machinery[3]. This guarantees that molecules vital for cellular function move efficiently[4]. RANBP9 has also interacted with key signaling molecules like ABL1 and SRC tyrosine kinases[5]. This influences downstream signaling events, which control cell proliferation, cell survival, etc.[6]. As shown in new research, RANBP9 could play a role in the pathogenesis of certain diseases[7]). Downregulation of circ-RANBP9 in laryngeal cancer and its clinical significance. 9(6). Cancer progression has been linked to the imbalance of the expression of RANBP9[7]). This is linked to cancer progression because the RANBP9 protein may contribute to deviated signaling pathways expressed in oncogenesis[8]. RANBP9 has also been involved in neurodegenerative disorders like Alzheimers, underlining the importance of cancer biology[7]).

RANBP9 has several functional domains facilitating interactions with proteins and their involvement in diverse signaling pathways[8]. The protein contains an N-terminal SPRY domain, which participates in protein-protein interactions and is important for binding to multiple protein targets. RANBP9 also has a LisH domain, which is implicated in microtubule dynamics and cellular trafficking[9]). The C-terminal region of RANBP9 has a CTLH domain, which aids in protein dimerization and oligomerization[9]). Additionally, RANBP9 contains a nuclear localization signal, enabling its translocation into the nucleus, where it can influence nuclear processes. RANBP9 commonly interacts with various signaling molecules, cytoskeletal elements, and transcription factors, making it significant in cellular homeostasis, signal transduction, and the regulation of gene expression. The adaptability of RANBP9 underscores its importance in maintaining cellular functionality and responding to physiological stimuli. RANBP9 functions to interact with other proteins in different types of interactions, such as RANBP9-cytoplasmic complexes, RANBP9-nuclear complexes, RANBP9-signaling complexes, and RANBP0-adhesion complexes. The formation of these complexes by the protein controls the NSCLC progression. RANBP9 (Ran-binding protein 9) has been implicated

in NSCLC due to its role in cell signaling, apoptosis, and tumor suppression. Reduced expression of RANBP9 has been associated with increased tumor growth, metastasis, and poor prognosis in NSCLC patients. It modulates pathways like EGFR and interacts with tumor suppressor proteins, influencing cell proliferation and survival. Therefore, targeting RANBP9-related pathways could offer potential therapeutic strategies for NSCLC treatment. The detailed mechanism of non-small cell lung cancer caused by RANBP9 dysregulation is shown in Figure 1. Cancer-causing external stimuli, such as ionizing radiation (IR) and chemotherapy, can induce DNA damage and mutations, which may inhibit RANBP9. The inhibition of RANBP9 leads to uncontrolled cancer cell proliferation, ultimately resulting in lung cancer.

Molecular docking is a popular computational tool to predict the interactions between small molecules and target proteins. [10]. By simulating these interactions, researchers can identify potential drug candidates that are likely to bind tightly and selectively to the target protein, which is crucial for the development of new therapeutic agents [11]. Molecular docking plays a significant role in the early stages of drug discovery by facilitating the screening of large compound libraries to identify lead compounds with the desired biological activity. Homology modeling is a technique that predicts the structure of a protein according to the amino acid sequence as well as the typical structures of related homologous proteins. [12] This method involves identifying a known protein structure with sequence similarity to the target protein, aligning their sequences, and making a protein structure utilizing the templates structure as a guide. The model is then refined and checked to ensure accuracy. Homology modeling is essential in functional annotation, drug design, protein engineering, and evolutionary studies, providing valuable insights when experimental methods are impossible. The current research uses computational (in silico) techniques to inhibit the RanBP9 protein. Computational research will help us screen thousands of compounds in a short amount of time compared to experimental validation. The experimental validation required extensive resources like machines, chemicals and expertise which is difficult to get. Therefore, a computational approach was used in this research. Moreover, the cost associated during the screening process will be a lot less compared to experimental screening. Finally, the ability to screen thousands of compounds using computational techniques will help in validating these compounds in a quick amount of time. Finally, the chemical compound selected from this research can be used for further experimental validation studies.

A scaffold protein is a helper protein that brings other proteins together to form a multi-protein complex assembly crucial for signal transduction and cell division [9]). In NSCLC tissues, RANBP9 is overexpressed compared to normal lung tissues [13]. Overexpression of RANBP9 means there is an increase in this protein production, resulting in cell proliferation (excessive cell growth) [13]. In addition, RANBP9 is involved in the regulation of cell cycle progression, promoting the proliferation of NSCLC cells [13]. Inhibiting these proteins could be a promising therapeutic strategy for NSCLC. We hypothesize that small molecules can potentially bind to this protein, restricting the RANBP9-mediated protein complex formation. In the present work, computational studies have been conducted to identify ligands that can interact with the RANBP9 and prevent the formation of protein complexes. As suggested by docking simulations, we have identified five ligands that exhibit a strong affinity for the protein. The druggable site of the protein (predicted by machine learning) and predicted by molecular docking were the same. Our research will help in designing novel therapeutics against NSCLC.

Results

RANBP9 is overexpressed in NSCLC, promoting cell proliferation and tumorigenesis. Its inhibition may offer a therapeutic strategy. Therefore, through molecular docking simulation, RANBP9 was used to predict inhibitors against the protein. The structure formed between RANBP9 and other complex proteins crucial for cell division is shown in Figure 2a. The predicted binding site obtained from the P2Rank web server shows the druggable site of the protein can inhibit the RANBP9, Figure 2b. It was also confirmed by molecular docking simulations that all the ligands bind to the druggable site, Figure 2c. There is a deep cavity at the site that takes in the ligands, Figure 2d. In this result the p21 protein was used as a control to further validate the results between as shown in Figure 2b and 2d. The p21 protein binds to the same draggable site of the protein which further validates that the predicted binding site and the draggable binding site are the same and could be a potential target site for the drug discovery.

As shown in Figure 3, the two-dimensional interaction image of the RANBP9-ligands portrays that most interactions were hydrophobic, with few observed hydrogen bonds. Figure 3 illustrates the interaction between the ligand and the protein. The ligand is shown in purple, while the amino acids involved in binding are represented in yellow or red eyelashes, indicating hydrogen bonds and hydrophobic interactions, respectively. In the protein structure, the hydrophobic nature of a deep cavity is common. Comparably, primarily hydrophobic amino acids surrounding the proteins were shown by the three-dimensional interactions image in Figure 4. Figure 4 shows that the ligand is pink, and the surrounding amino acids are shown in cyan color. The PLIP software was used to predict the interactions between RANBP9 and ligands, as shown in Table 1, Figure 5. Based on PLIP analysis, Ligand I formed three hydrophobic bonds with Tyr229, Leu244, and Trp247 at a distance of 3.46, 3.47, and 3.41 , respectively. Furthermore, two hydrogen bonds with His255 (2.77) and Phe 262 (2.86) were also observed

in the complex. Ligand II, III, IV, V, and VI. Four hydrophobic interactions formed by the Ligand II consisting of Tyr229, Leu244, Trp247, and Gln319 at distances of 3.90, 3.32, 3.80, and 3.64 , respectively. The Ligand III formed four hydrophobic interactions with Ile214, Tyr216, Phe293, and Phe293 again at a distance of 3.79, 3.75, 3.66, and 3.75 , respectively. Moreover, one hydrogen bond with Asn286 at a distance of 3.01 was also observed in the complex. Ligand II, III, IV, V, and VI. There were five hydrophobic interactions formed by the Ligand IV consisting of Ile214, Ile214, Ile214, Tyr216, and Pro333 at distances of 3.91, 3.93, 3.75, 3.80, and 3.75 respectively. There was also a hydrogen bond with His332 at a distance of 2.55 . The Ligand V formed three hydrophobic bonds with Tyr229, Tyr229, and Gln319 at 3.80, 3.57, and 3.51 , respectively. There were five hydrophobic bonds formed by the Ligand VI consisting of Tyr229, Tyr229, Leu244, Tyrp247, and Gln319 at distances of 3.80, 3.57, 3.51, 3.93, and 3.62 respectively. Two hydrogen bonds were observed at 2.08 and 2.83 , respectively, with Asp258 and Gln319. The hydrogen bonds are much stronger than the hydrophobic bonds, and based on these simulations, ligand IV formed a higher number of hydrogen bonds and hydrophobic interactions combined; therefore, it was selected as an appropriate candidate.

The SwissADME software was used to compute the pharmaceutical properties of the six ligands, Table 2. All six ligands showed a high GI absorption, indicating how well a drug is absorbed from the gastrointestinal tract to the bloodstream. The drugs capacity to pass the blood-brain barrier and control the CNS is represented by BBB permeation. Of all the six ligands, only ligands II, III, IV, and VI can pass the blood barrier. Lastly, Lipinski (drug-likeliness) gauges whether a compound has properties that show it is an orally active compound. This is based on Lipinskis Rule of Five. Looking at this rule, we can determine that all six ligands pass the Lipinski rule of five. Drug-likeness was computed to quantitatively analyze whether a compound possessed the necessary pharmaceutical properties to be active and function as a drug in humans. Various factors, such as molecular weight, solubility, lipophilicity, hydrogen bond ability, and structural complexity, were evaluated based on Lipinskis Rule of Five. Drug-likeness assessment played a crucial role in early-stage drug discovery by filtering out compounds that were more likely to be promising drug candidates. The computation of lipophilicity was conducted to determine whether the drug met the required criteria, further confirming that the ligand had a higher chance of being a viable drug candidate.

Discussion

Ran Binding Protein 9 (RanBP9) is a multifunctional cascade protein playing an important role in cancer development by regulating cell proliferation, apoptosis, and metastasis. Initially, the protein was linked with age-related diseases such as Alzheimer's disease; however, later, the protein's relation to cancer was also discovered. Dysregulation of the protein is closely linked to NSCLC. Recently, the relationship of RANBP9 and cellular response to DNA damage has been studied by Matsuoka et al. [14]. Later, Palmieri et al also showed that Ataxia Telangiectasia Mutated (ATM) kinase (a critical enzyme involved in the cellular response to DNA damage) and RanBP9 protein are involved in (DNA damage response) DDR in NSCLC [8]. This study reveals a novel role for Ran Binding Protein 9 (RanBP9) in the DNA damage response of lung cancer cells, challenging previous research that linked it mainly to cell signaling and transport. This finding suggests a potential new target for lung cancer therapy.

In their study they have explored the effect of DNA damaging agents (like ionizing radiation (IR) and cisplatin) on the NSCLC cells and found that the protein exhibits an increase in sensitivity to both IR and cisplatin. They also found that RANBP9 is highly expressed in NSCLC cells in comparison to other lung tissues.

Based on these results, we have identified that RanBP9 is a protein that forms complexes with other proteins and is involved in cell prolif16eration and cancer genesis. Therefore, inhibiting RanBP9 from forming complexes with other proteins could potentially inhibit non-small cell lung cancer. The first result shows that the druggable site of RanBP9 and the molecular duct structure were identical. A careful evaluation of the druggable site reveals that it has a cavity, which could be crucial for protein binding. We have also docked ligands and the p21 protein, which is involved in cell proliferation, and found that both the ligand and the p21 protein bind to the same druggable site. Therefore, blocking this binding site could potentially inhibit the function of RanBP9. A careful evaluation of another result shows that the ligands are mostly hydrophobic in nature and form hydrophobic interactions with the surrounding protein. Finally, based on the number and strength of interactions, we have identified ligand number 6 as the most appropriate candidate. We also computed the predicted pharmaceutical properties of these ligands and found that all ligands have high gastrointestinal (GI) absorption. Therefore, these ligands exhibit high water solubility and could be viable as oral drugs. Additionally, the selected ligand 6 showed high blood-brain barrier (BBB) permeability, which is a measure of the drug's ability to reach the brain. Finally, all the drugs have demonstrated high drug-likeness, indicating that these results are promising, as there is a high likelihood of these drugs becoming viable drug candidates.

RANBP9 protein also has a highly homologous protein RANBP10 which can potentially function in full or partially in the absence of RANBP9. To test the effect of both proteins, experiments by deleting both RANBP0 and RANBP10 will put light on this question. In addition, the RANBP9 protein has also been suggested as a potential biomarker of identifying the possible therapies for cancer and the druggable site found in this research can also be used for ligand binding site [13].

There are some limitations of this research, however. The

molecular dockings quality depends largely on the precision of the models and algorithms utilized and imprecise models can result in false positives. Accordingly, we tried to justify our docking protocol by laying over the experimentally discovered structure and docked structure and got an RMSD of 1.4A. However, to justify the computational research to a greater extent, post computational analysis like SPR (surface plasmon resonance) or ITC (isothermal calorimetry) should be executed. These studies will further justify that the suggested ligands are binding to the protein. Additionally, a comprehensive binging analysis needs site directed mutagenesis and co-crystallized structure will exhibit the amino acids involved in binding. In future studies, biophysical techniques such as surface plasmon resonance will further enhance our protein-ligand interactions. Understanding in real-time binding kinetics, it will help us give the binding affinity Kd value which will also further validate that ligand binds to the protein. In addition, isothermal calorimetry, the heat exchange during the ligand binding can also be measured.

Using computational docking simulations, this work found putative inhibitors of Ran Binding Protein 9 (RanBP9), a key factor in the development of NSCLC. High binding affinities were demonstrated by six ligands; however, ligand VI stood out as the most viable option because of its high hydrogen bonding and hydrophobic interactions. Positive drug-like characteristics, such as strong gastrointestinal absorption and blood-brain barrier permeability, were validated by SwissADME research. These results demonstrate RanBP9's potential as a therapeutic target, providing a basis for upcoming experimental confirmation and the creation of focused NSCLC therapeutics.

Materials and Methods

Protein modeling: The amino acid sequence of RanBP9 was obtained from UniProt web server and the amino acid sequence was used. The three-dimensional structure of the RANBP9 protein was predicted by using AlphaFold 3.[15] Prediction was validated by further prediction were validated by docking the predicted co-crystallized structure of the protein which shows an RMSD of 1.8.

Ligand selection: The ligands were obtained from Zinc-20 database in the trenches section.[16]. Since Zinc-20 database has millions of compounds, we have used the following criteria to decrease the number of ligands. First, only the 3D models were selected. In the next section, REACT only standard and exclusive was selected. In PURCH section, in stock and exclusive was selected. The pH was referenced and only neutral compound with charge 0 were selected. Finally, lead like compounds were selected. Lead compound is a biological respective of a chemical compound that has a potential to progress and become a full drug in a drug development program. It is based on the potency, selectivity, pharmacokinetic and physicochemical properties of the ligands. The log p value was selected because the oral drugs have a log p value of close to 2. Therefore, the log p value was selected. In addition, the reason log p value selected 2 is to decrease the number of ligands which can be scanned. Lead like is a selection criterion in zinc database which follows specific criteria for instance molecular weight should molecular weight log P value hydrogen bond donor hydrogen bond acceptor and rotatable bonds are selected for the further processing. This helped us get to a very low number of ligands. Finally, chemical compounds with log p value of 2 were selected because log p value of 2 have higher chances of being oral drugs. The following criteria helped us to get 5000 ligands for molecular docking simulation. Based on these selection criteria nearly 5000 compounds were chosen for virtual screening in the Zinc20 database, which was used to acquire the ligands for molecular docking. Compounds that exhibit favorable oral and intestinal absorption are chemical compounds and were picked for the next step.

Molecular docking simulation: The AutoDock Vina 1.5.6. software was employed to examine the protein-ligand binding configurations. The AutoDock Vina was used in the default parameters for example exhausted exhaustiveness was set to 8. Grid box parameters size was the center was ($center\_x = -23.693$, $center\_y = -61.307$, $center\_z = 16.644$) and size was 40, 40, 40 scoring function empirical scoring function was used and random seed was random was selected for molecular docking simulation. Number of modes = 10 and energy range was set to 4. To further validating the docking validated was cross referenced cross checked by using HDOCK software and the docking simulation showed similar results which means the top six ligands were selected by HDOCK software and the predicted binding site remained the same.

Molecular docking analysis: To predict the druggable site on the RANBP9 protein, a machine learning tool, P2rank, was used. P2rank is used to predict protein-ligand binding sites. P2rank uses a machine learning algorithm to predict the ligand-binding sites on the structures of proteins based on the properties of the protein's surface. P2rank is very useful for computational drug discovery and other applications in bioinformatics and structural biology as it is designed to be very fast and accurate. The molecular talking simulation was analyzed by first by comparing the draggable binding site on the protein. In addition, a two-dimensional picture of the ligand was obtained by LigPlot software.[17]. It shows the amino acid, and which are surrounding the ligand. The Figure is shown in Figure 3.

In addition, the three-dimensional structure of the ligand is shown in Figure 4 and was obtained from ChimeraX[18]. A final analysis was done by using a protein ligand interaction profiler in which the number of interactions formed between the protein and the six ligands is shown. All the ligands form hydrophobic and hydrogen bond interactions.

Finally, the pharmaceutical properties of the selected six ligands were obtained. It was obtained by Swiss ADME web server

which calculates the GI absorption, the amount of drug that the drug is can be easily absorbed by stomach or not, blood-brain barrier permeation, the drug is permeable to blood-brain barrier and finally the drug likeliness Lipinski, that does the drug passes the Lipinski rule of five.[19]. The following parameters are shown in table 2.

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Appendix

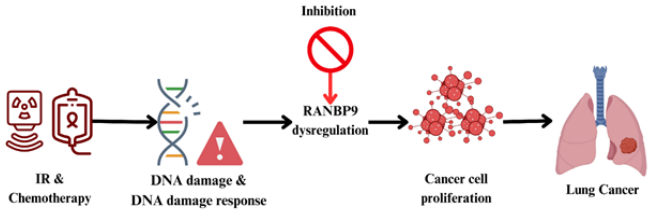

Fig. 1 Progression of lung cancer. In the presence of external stimuli (IR and chemotherapy) the damage in the DNA takes place which activates the DNA damage response. Dysregulation in the RANBP9 protein leads this into cancer cell proliferation and eventually leads to lung cancer.

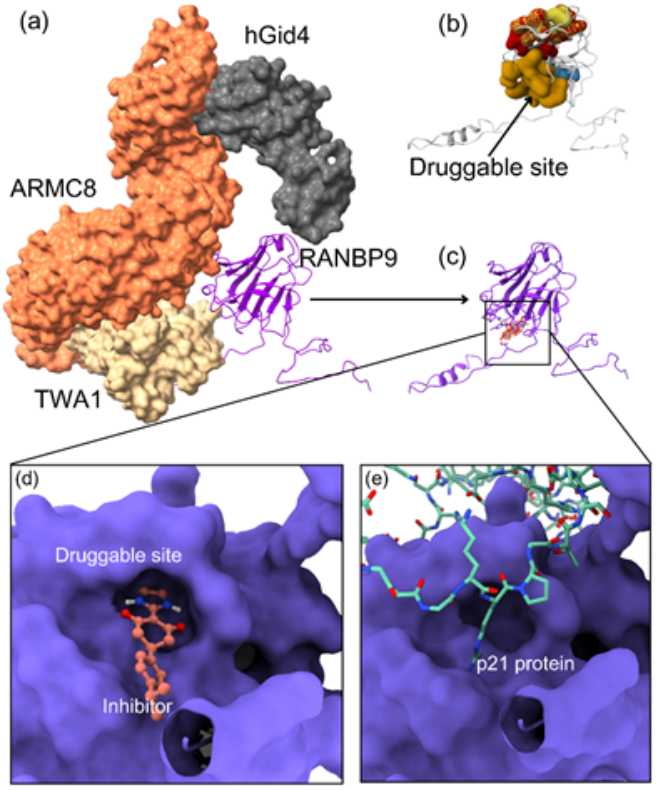

Fig. 2 RANBP9 structure. (a) RANBP9 (purple) is a multifunctional scaffold protein that interacts with a variety of proteins and is involved in multiple cellular processes; (b) According to graph neural network (GNN) the druggable site is shown in yellow; (c) Based on molecular docking the ligands also bind to the druggable site predicted by GNN; (d) All the ligands bind to the cavity shown in image; and (e) p21 protein also binds to this cavity.

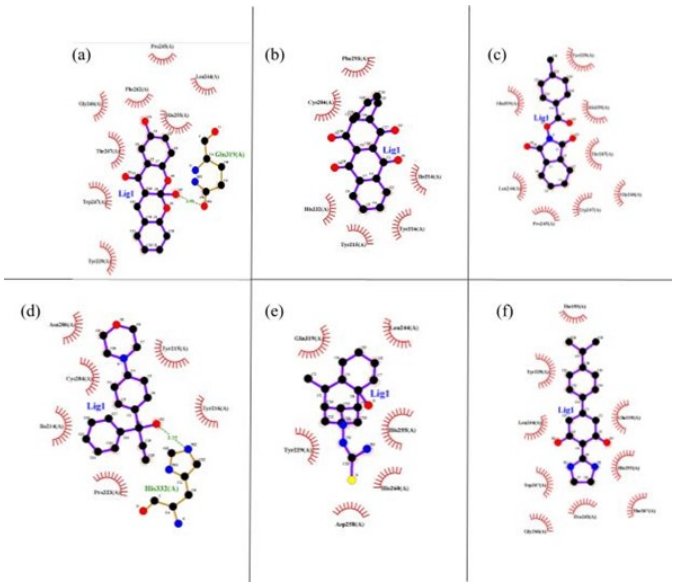

Fig. 3 Two-dimensional pictures of ligands and the interacting amino acids of the protein. The figure shows that most of the interactions are hydrophobic in nature. The ligand is shown in purple color, hydrophobic interactions are in red eyelashes, and green lines are hydrogen bonds.

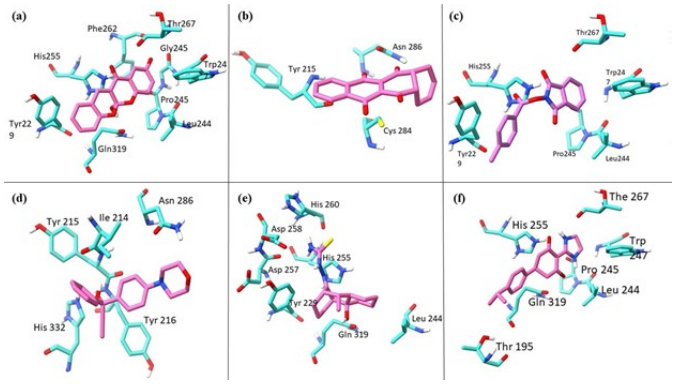

Fig. 4 3-dimensional pictures of ligands and the interacting amino acids of the protein. The ligand is shown in pink color.

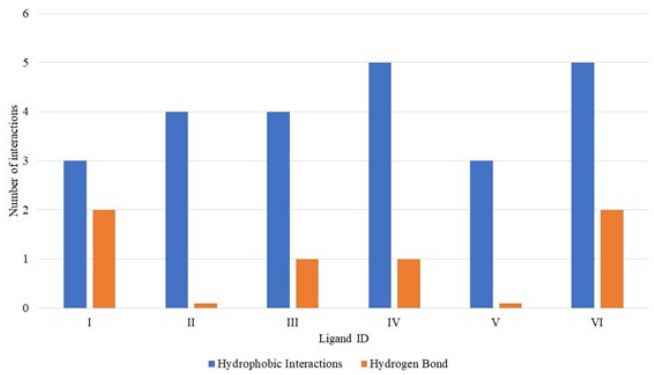

Fig. 5 Number of Interactions formed in the ligands.

Table 1 Table showing the protein-ligand interactions

Ligand I		
Hydrophobic Interactions	Residue	Distance ()
	Tyr229	3.46
	Leu244	3.47
	Trp247	3.41
Hydrogen Bond	His255	2.77
	Phe262	2.86
Ligand II		
Hydrophobic Interactions	Tyr229	3.9
	Leu244	3.32
	Trp247	3.8
	Gln319	3.64
Ligand III		
Hydrophobic Interactions	Ile214	3.79
	Tyr216	3.75
	Phe293	3.66
	Phe293	3.75
Hydrogen Bonds	Asn286	3.01
Ligand IV		
Hydrophobic Interactions	Ile214	3.91
	Ile214	3.93
	Ile214	3.75
	Tyr216	3.8
	Pro333	3.75
Hydrogen Bonds	His332	2.55
Ligand V		
Hydrophobic Interactions	Tyr229	3.69
	Tyr229	3.6
	Gln319	3.76
Ligand VI		
Hydrophobic Interactions	Tyr229	3.8
	Tyr229	3.57
	Leu244	3.51
	Tyrp247	3.93
	Gln319	3.62
Hydrogen Bonds	Asp258	2.08
	Gln319	2.83

Table 2 Pharmaceutical properties of proposed ligands. GI absorption: refers to how well a drug is absorbed from the gastrointestinal tract into the bloodstream; BBB permeation: indicates a drug's capacity to influence the central nervous system by crossing the blood-brain barrier, drug-likeness (Lipinski): assesses whether a compound has properties that would make it a likely active drug in humans, based on Lipinski's Rule of Five.

	I	**II**	**III**	**IV**	**V**	**VI**
Formula, molecular weight	C16H10O5 282.25 g/mol	C18H12O4 292.29 g/mol	C16H11NO4 281.26 g/mol	C19H19NO2 293.36 g/mol	C15H25N3OS 295.44 g/mol	C18H22N2O2 298.38 g/mol
GI absorption	High	High	High	High	High	High
BBB permeation	No	Yes	Yes	Yes	No	Yes
Drug likeliness (Lipinski)	Yes	Yes	Yes	Yes	Yes	Yes
2D structure