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Designing an Inhibitor Molecule to Combat Cancer through the Inhibition of Mutant PI3K (P110 α) Subunit Protein

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Abstract

The PI3K pathway plays a crucial role in the development of various types of cancers. Specifically, the P110 α component is frequently modified in the formation of human cancer, leading to disruptions in cell growth regulation and the emergence of malignancies. This research paper delves into the increasing utilization of computational tools to forecast potential molecules capable of inhibiting cancer. To validate stable structures of PI3K (P110α) component protein models, the study employed Ramachandran map analysis, which revealed active regions in close proximity to the mutation site. Through the employment of PyRX software, molecular docking studies were conducted, resulting in the identification of four inhibitor derivatives that exhibited significant effectiveness with the lowest docking energies in preventing the mutated PI3K (P110 α) component protein structures. Furthermore, these inhibitors adhered to Lipinski's rule of five, further enhancing their potential.

Keywords: PI3k; Modeller; Molecular; Docking; PyRx

INTRODUCTION

The proto-oncogene PI3K (Phosphoinositid-3-Kinase) controls various essential cell signaling pathways that stimulate cellular replication and proliferation while repressing growth and apoptosis (Cantley 2002; Moens et al., 2013). The PI3K protein family comprises eight members categorized based on their sequence, domain structure, and mode of regulation into three groups: Class I PI3K, Class II PI3K, and Class III PI3K. Class I PI3K is composed of p110 α, p110 β, and p110γ subunit classes and plays a significant role in cancer biology. PI3K mutations are prevalent only in Class I PI3K, with the p110α subunit being the most commonly mutated protein in breast, endometrial, urinary tract, colorectal, and ovarian cancers. Mutations at codon 542, 545, and 1047 of PI3K (P110α) subunit protein are the most frequent mutational events in human carcinogenesis, resulting in the constitutive activation of downstream pathways and altered cellular proliferation and malignant transformation regulation. All this makes it an attractive drug target for treating cancer. Validation and identification of disease-associated targets are crucial for drug design and discovery. Mutations in the PIK3CA gene(s) that code PI3K (P110α) subunit promote uncontrolled cell growth and proliferation while inhibiting apoptosis. Chemotherapy, operative intervention, radiation treatment, and hormone therapy are conventional methods for managing cancer. However, they lack specificity and often damage healthy cells, resulting in side effects. Target-based drug design offers a promising approach to utilizing the PI3K (P110α) subunit as an anticancer protein (Jorgensen, 2004; Pearce

et al., 2008; Sliwoski et al., 2013; Bassani and Moro 2023). Recent studies have demonstrated the efficacy of targeting the PI3K (P110α) subunit in cancer therapy (Apel et al., 2019; Uniyal et al., 2022; Sen et al., 2022).

MATERIALS AND METHODS

A Boolean query was used to trace the protein structure of PI3K (P110 α) subunit in Homo sapiens and was analyzed against the UniProt database (http://www.uniprot.org). The protein sequence query (P42336) was obtained in fasta format from the UniProt database (http://www.uniprot.org/uniprot/). Modeller 9.17 software was utilized to predict the 3D structures of both wild-type and mutated proteins (Sali et al., 1995). Relevant mutations at codons 542, 545, and 1047 of the PI3K (P110 α) subunit were collected from literature sources (Engelman et al., 2006; Yuan and Cantley 2008; Janku et al., 2011). The quality of the structural models was assessed using the SAVESv6.0 tool, which employs the Ramachandran plot to evaluate the stereochemical characteristics of the protein structure (Laskowski et al., 1993). The predicted active site of the mutated conformations of the PI3K (P110 α) subunit was determined using PASS software and utilized for virtual screening. In silico virtual screening was performed using PyRx software to rank the library molecules based on their affinity towards the active site of the mutated protein conformations of the PI3K (P110 α) subunit. The top ten potential library molecules were further analyzed using Lipinski filter software to assess their suitability as drugs (Lipinski et al., 1997). Recent studies have demonstrated the effectiveness of in silico virtual screening in the field of drug discovery (Patra et al., 2014; Garner et al., 2017; An et al., 2020; Kim et al., 2022; Saad and Almabruk 2022).

RESULTS AND DISCUSSION

In this study, we utilized the homology modeling procedure to establish the arrangement of the PI3K (P110 α) subunit protein along with its three modified models. This allowed us to gain a better understanding of the structural organization of these proteins and their potential implications. The BLASTP search was performed against the PDB database using the Modeller 9.17 software to find suitable templates for homology modeling. The template with maximum identity, high score, and lower e-value was selected for homology modeling. The final stable structure of the PI3K (P110 α) subunit protein was visualized in Figure 1.

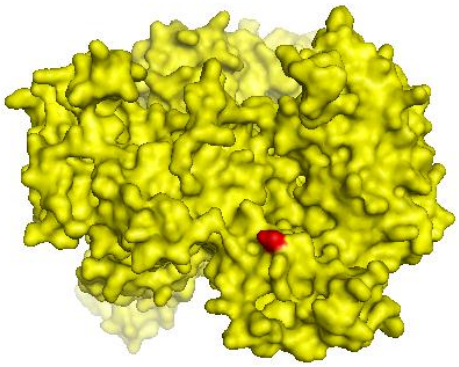

Figure (1). Protein models constructed for wild type with normal codon 545 (Glu) in red color.

The modifications were selected based on the findings of several prior studies, including those conducted by Samuels et al. in 2004, Lee et al. in 2005, Ikenoue et al. in 2005, Isakoff et al. in 2005, Kang et al. in 2005, Samuels and Ericson in 2006, and Zhao and Vogt in 2008. These findings provide valuable insights into the structural aspects of PI3K (P110 α) subunit protein and its mutations, which can be beneficial in drug discovery and design. Recent studies have demonstrated the potential of homology modeling in drug discovery (Saad 2017; Saad and Attialla 2017; Kim et al., 2022; Samad et al., 2023; Osweiher and Saad 2022). Additionally, homology modeling has been used to determine the structure of various proteins involved in diseases such as cancer and infection by SARS-COV-2 (Apel et al., 2019; Sen et al., 2022). The use of mutated models in homology modeling has also been shown to be an effective approach to drug discovery (Uniyal et al., 2022; Samad et al., 2023).

In this study, the homology modeling procedure was used to determine the arrangement of the PI3K (P110 α) subunit protein and its three modified forms. For each protein, three distinct models was created. Figures 1 and 2 showcase the selected models for the normal and mutated 545 codons of the PI3K (P110 α) subunit. These figures clearly demonstrate the significant alterations in amino acids caused by the mutations in codon 545.

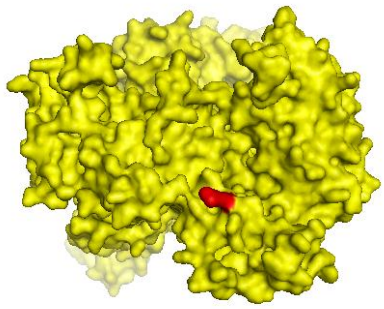

Figure: (2). Protein models constructed for mutated PI3K (P110 α) subunit of Codon 545 which mutated by Lys in Red color.

In this investigation, comparative modeling techniques were used to create protein structures of both the natural and mutated forms of the PI3K (P110 α) subunit. The resulting models were then evaluated using the SAVES server from the NIH MBI Laboratory for Structural Genomics and Proteomics. Two tools, PROCHECK and Verify_3D, were employed to assess the validity of the models. Interestingly, one of the models generated by Modeller was found to be more acceptable than the others, making it the most reliable choice for each protein. This selection was made based on Table 1.

When analyzing the protein models, it was found that the majority (85.1%) of the phi/psi angles of the residues fell within the most favored regions. This indicates that the models possess a high level of quality. To identify potential binding sites within the protein models, the researchers utilized PASS software. As a result, three potential binding sites were identified. To pinpoint the active site, the researchers considered the probe with the largest number of residues. These findings provide valuable insights into the structure of the PI3K (P110 α) subunit protein, as well as its mutation. Such information can prove to be highly advantageous in the field of drug discovery and design.

Table: (1). Validation of PI3K (P110 α) subunit structure

Selected PI3K (P110α) protein model	Ramachandran map analysis			
	phi/psi angles %	Additional allowed regions	Additional disallowed regions	Generously allowed regions
Model of PI3K (P110α)	85.1	11.7	0.8	2.4

In various studies, the quality of protein models has been assessed using validation tools like PROCHECK and Verify_3D (Colovos and Yeates, 1993; Berman et al., 2000; Lovell et al., 2003). These evaluations have shown that software such as PASS is effective in identifying binding sites in protein models for drug discovery (Saad and Attialla 2017; Apel et al., 2019; Sen et al., 2022; Osweiher and Saad 2022; Samad et al., 2023). The main aim of this investigation was to compare the binding sites detected by PASS software with the active site of the template protein in order to identify the active site of PI3K (P110 α) subunit protein models. The results revealed that the active site was located at residues 570, 596, 616, 631, 636, 676, 686, and 885, as shown in Figure 3.

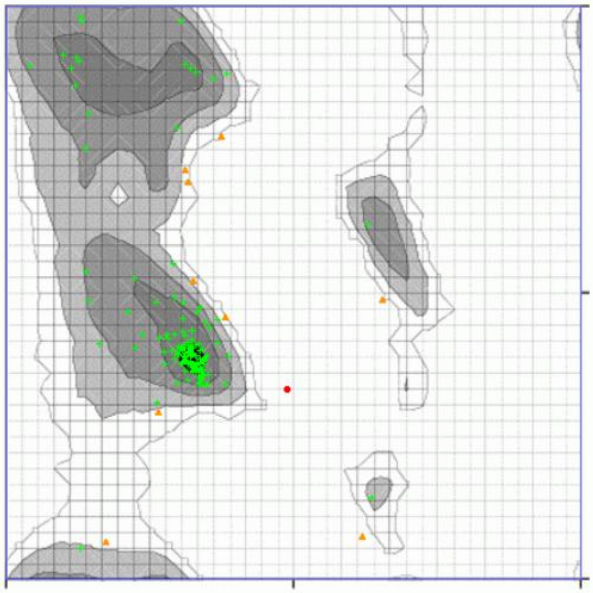

Figure: (1). Shows a Ramachandran plot of PI3K (P110 α) subunit wild-type protein models to validate the structure.

These identified active site residues align with those previously reported in other studies as part of the active site (Shah et al., 2002; von Bubnoff et al., 2005; Zunder et al., 2008). Furthermore, ten potential inhibitor molecules were screened and found to target the active site in the mutant codon 545 of the PI3K (P110 α) subunit protein. This approach provides valuable insights into the structure of the PI3K (P110 α) subunit protein and its potential inhibitors, which can considerably aid the drug discovery and design process. Recent studies have demonstrated the usefulness of computational approaches in drug discovery, such as virtual screening and molecular dynamics simulations (Cheol-Min et al., 2006; Apel et al., 2019; Ton et al., 2020). The use of software such as PASS to identify active sites in proteins has also been shown to be effective in drug discovery.

This analysis utilized molecular docking to identify potential inhibitor molecules for the PI3K (P110 α) subunit protein which is presented in Table (2). The identified inhibitors were found to be in agreement with previous docking studies conducted by Garcia-Echeverria and Sellers

(2008); Saad (2017); Saad and Attialla (2017) and Jin et al., (2021). Additionally, the potential inhibitors met the criteria of Lipinski's 'rule of five', which evaluates drug-likeness based on properties such as solubility, partition coefficient, and molecular weight. These findings suggest that the identified inhibitors have the potential to be effective drugs for PI3K (P110 α) subunit protein and can be considered for further research. Recent studies have demonstrated the importance of drug-likeness evaluation in drug discovery, such as Lipinski's 'rule of five' (Bickerton et al., 2012; Pires et al., 2015; Saad 2017; Saad and Attialla 2017). Moreover, the use of computational methods such as molecular docking has been shown to be effective in the identification of potential inhibitors for various proteins (Muryshev et al., 2003; Cheol-Min et al., 2006; Pinzi and Rastelli 2019; Khan et al., 2022).

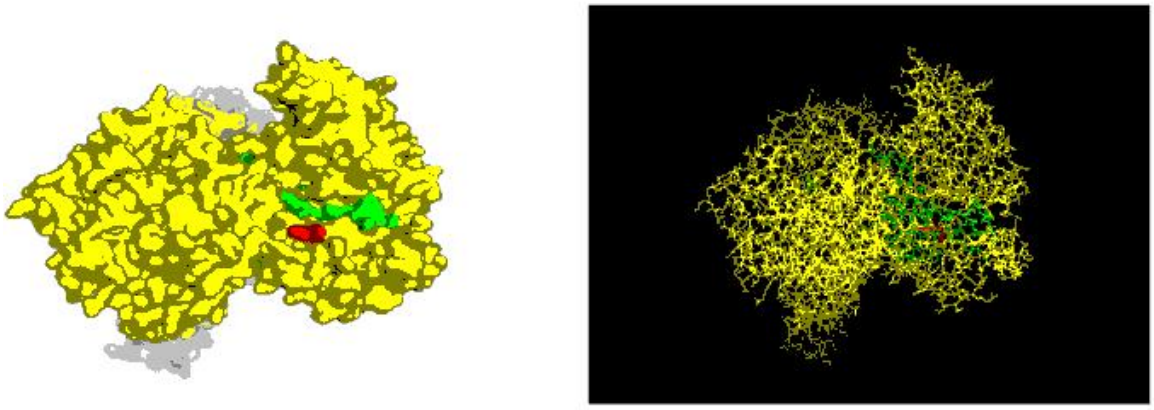

Figure: (3). The active site was identified by PASS green colors show Point mutations at codon 545 red color show active site point of PI3K (P110 α) subunit

Table (2). Docked Energy and Lipinski's Values of Ligand Molecules

Ligand molecules	Molecular formula	Docking energy (kcal/mol)	Xlog P<5	H-Bond donor <5	H-Bond Acceptor <10	Molecular weight (g/mol) <500
Sorafenib	C21H16ClF3N4O3	-12.90	4.200	3	7	464.50
Pilaralisib	C21H16N6O2S2	-11.30	0.4303	0	2	443.00
vemurafenib	C23H18ClF2N3O3S	-10.01	2.6878	1	3	466.00
BEZ235	C30H23N5O	-9.10	-0.6025	1	1	463.00

CONCLUSION

By conducting computational analysis and examining available experimental data, we have identified the most effective ligand molecules for inhibiting the function of the mutant PI3K (P110 α) subunit protein. We specifically focused on ligands that adhere to Lipinski's 'rule of five'. These molecules have demonstrated the ability to halt the growth and proliferation of cancer cells, making them highly promising candidates for future drug development and research endeavors.

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