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In-silico designing of a potent ligand molecule against *PTEN* (Phosphatase and tensin homolog) implicated in Breast Cancer

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ABSTRACT

Breast cancer has been attributed to be the second most common malignancy in females worldwide after skin cancer associated with a significantly high mortality rate. Tumor suppressor genes have an indispensable role in maintaining genomic integrity as well as cell cycle regulation. Phosphatase and tensin homolog deleted on chromosome ten (*PTEN*) is one of the most frequently mutated human tumor suppressor genes, implicated in cell growth, survival, and suppressing tumor formation. As the tumor progresses to more advanced stages, genetic alterations tend to increase one such alteration is the mutation of the *PTEN* gene which is linked to programmed cell death and maintenance of cell cycle regulation. There is a syndrome known as Cowden syndrome associated with a high risk of breast cancer which is a result of an outcome of germline mutations in the *PTEN* gene. Loss of *PTEN* activity, either at the protein or genomic level, has been related to many primary and metastatic malignancies including breast cancer. This study focuses on developing a potential bioavailable ligand inhibitory molecule for *PTEN*, using a computer-aided drug design approach (CADD). A library of developed ligands consisting of 50 potential molecules was screened to find a potential candidate to be used for second generation drug development. Among them, LIG28 was adjudged as the most effective and potential *PTEN* inhibitor given its maximum binding affinity of ΔG -5.96Kcal/mole with a lower RMSD value. Carmer's Rule of toxicity further revealed the compatibility and non-toxicity of the molecule. These observations underscore the importance of *PTEN* as a target in the development of tumorigenesis and the prognosis of breast cancer.

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1 Introduction

Cancer is attributed to the unregulated and uncontrolled division of cells, which may become malignant and spread into the neighboring tissues of the body. Genetic changes are encountered at the DNA level when cancer becomes malignant (Rajpoot et al. 2021). In the current scenario, breast cancer is known to affect one in every eight women during their life span worldwide (Momenimovahed and Salehiniya 2019). In recent times various promising inhibitors have been developed for the treatment of breast cancer targeting various molecules including mTOR and *PTEN* (Sharma et al. 2020a). *PTEN* was considered as an autonomous anticancer unit (Trotman and Pandolfi 2003; Sharma et al. 2021a,d) which acts as a tumor suppressor by antagonizing the PI3K and AKT pathways and plays an important role in cell survival, cell migration, cell and organ size control (Barbieri and Rubin 2015).

PTEN despite being called Phosphatase Tensin Homologue is also reported to be mutated in Multiple Advanced Cancer1 (MMAC1) located at chromosome 10q23.3. It encodes for 403 amino acids which display both lipid as well as protein phosphates activities. In *PTEN*, there are two functional domains (phosphate domain and a C2 domain) along with three structural regions {a short N-terminal phosphatidylinositol (PI)-4,5-bisphosphate (PIP2) binding domain and a C-terminal tail containing PEST sequences and a PDZ-interaction motif} (Sharma et al. 2016). *PTEN* acts as a negative regulator of the PI3K/AKT signaling pathway affecting cell survival, proliferation, and apoptosis directly and indirectly (Sharma et al. 2017; Ma et al. 2019). In the inositol ring, *PTEN* dephosphorylates at the 3' end of the triphosphate (PIP3) resulting in (PIP2) biphosphate, which obstructs AKT activation and downstream signaling processes (Sehrawat et al. 2021). Lack of inhibition of the AKT-dependent processes and inactivation of *PTEN* has been related to tumorigenesis in multiple human cancers (Sharma et al. 2019a), including breast cancer (Roy et al. 2010; Ram et al. 2020). During intracellular signaling, recruitment of AKT initially depends on the generation of phosphatidyl-inositol-triphosphate (PIP3) by PI3K stimulated through receptor-coupled tyrosine kinases (RTKs) (Hinz and Jücker 2019; Sharma et al. 2022a). Engagement of PIP3 to AKT leads to double phosphorylation one on the kinase domain (T308, T309, and T305 for AKT1, 2, and 3) by PDK1 and the other one on a regulatory domain (S473, S474, and S472 for AKT1, 2, and 3,) by mTOR complex 2 (Sehrawat et al. 2021). Upon activation, AKT phosphorylates its downstream targets, including tuberous sclerosis complex 2 (TSC2) (Sharma et al. 2022b), glycogen synthase kinase-3β (GSK3β), and the forkhead kinase transcription factors (FOXO), thus help in increasing cell proliferation, metabolism, and survival (Hoxhaj and Manning 2020).

It has been observed that *PTEN* emerges as a suppressor of breast cancer growth by down-regulation of PI3K which results in cell death and arrest of the G1 phase of the cell cycle. Protein structure shows a phosphatase domain that contains 1-185 residues and a C2 domain having residues 186-351 both essential for tumor suppressor function (Sharma et al. 2022c). The phoshatase domain contains the tyrosine phosphatase signature motif (H123CKAGKGR130), which forms a loop (P-loop) with the active site pocket. Inside this loop residue, C124 and R 130 are important for catalysis (Sharma et al. 2019b). *PTEN* is phosphorylated on a group of serine and threonine residues present on its C-terminal tail and results in a closed *PTEN* state in its inactive form (Singh et al. 2022) and maintains *PTEN* protein in a fixed conformation (Sheikh et al. 2020). Upon activation, the phosphatase domain of *PTEN* opens up by the de-phosphorylation of its C terminal tail, which results in increased activity of *PTEN* (Panwar et al. 2021). Considering the significance of *PTEN* in breast cancer development, prognosis, and treatment (Sharma et al. 2020b), CADD was exploited to develop a potential bioavailable ligand inhibitory molecule for *PTEN*. A library of developed ligands consisting of 50 potential molecules was screened to find a potential candidate to be used for second generation drug development (Sharma et al. 2021b,e).

2 Materials and Methods

NCBI, UniProtKB, PROCHECK, and PROSA were used to retrieve and validate the *PTEN* protein sequence. The information on the structures for *PTEN* was extracted using PDB. The models for the parent protein have been designed using iterative threading refinement (I-TASSER) (Roy et al. 2010; Zhang and Yu 2010; Yang and Zhang 2015;) program (Table 1). The designed models were then refined using PROCHECK (Wiederstein and Sippl 2007), which revealed model 1 as the most stable structure with significant core residues (Figure 1A). Further, the complete quality examination by Z- score (-12.46) indicated that the obtained model was significantly close to the template (Sharma et al. 2021c). As per the literature review, Histidine (HIS) at 123 positions, Lysine (LYS) at 125 positions, Glycine (GLY) at 127 positions, and Lysine (LYS) at 128 positions) formed the active site (Figure 1B) (Sharma et al. 2020c).

Seed molecule was explored using NCBI pub-Chem, which showed 4-Nitroquinoline as a natural ligand of the crystal structure of the *PTEN* tumor suppressor. The compound exhibits an idle molecular weight i.e 190.16 g/mol and it has the natural tendency to bind with phosphate inositol. The seed molecule was generated using Chemsketch Acd Labs (Figure 1C). The generated seed molecule was taken to AutoDock Vina autodock 4.2.6 which shows the best-fitted space of 4-Nitroquinoline close to binding tetrads. The seed molecule was positioned to interact favorably with the site (Figure 1D).

Table 1 Modelled Protein structure information with Template using ITASSER

Sr. No.	Model No.	Ramachandran Plot			Bad Contacts	G - Factor
		Core	Allowed	Disallowed		
1.	Rag 1	80.3%	15.6%	1.4%	5	-0.34
2.	Rag2	77.3%	17.7%	1.8%	31	-0.56
3.	Rag3	78.0%	16.1%	2.5%	23	-0.51
4.	Rag4	79.8%	16.0%	2.0%	5	-0.38
5.	Rag5	78.8%	14.6%	3.0%	27	-0.45

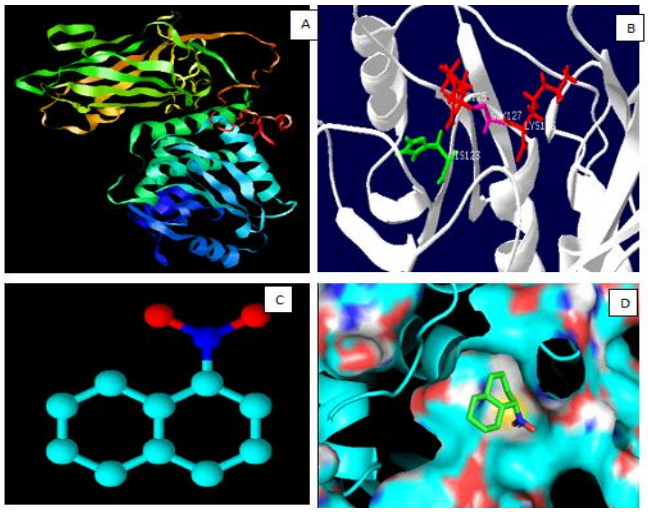

Figure 1 Predicted the best model (A) with Active site residues (B) 4-Nitroquinoline Structure (C) best binding confirmation of the lead molecule (D)

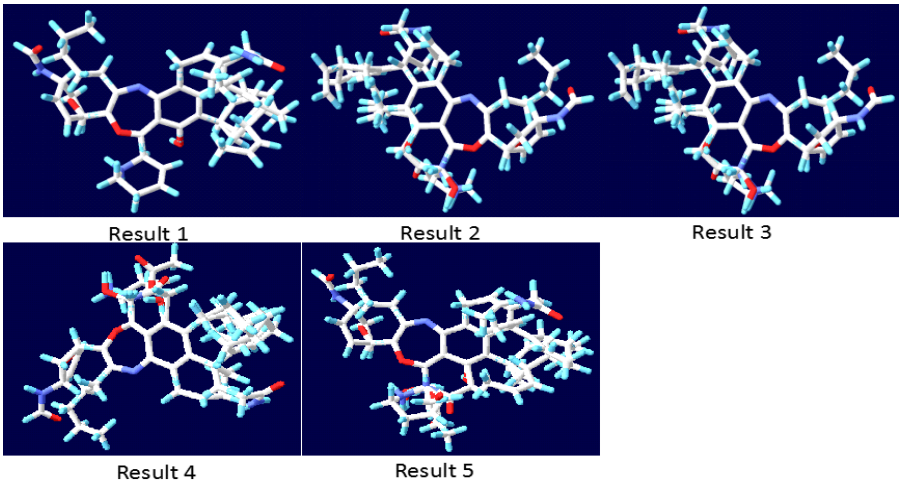

Figure 2 Best five Results obtained using the Ligbuilder

To achieve structural harmony of the site of interest, a complex of seed and target (Fused Space File) was used for fragment addition. More than 500,000 molecules were occupied by using an inbuilt library of organic fragments and a Genetic Algorithm under the Growing strategy of Ligbuilder (v1.2) which was used to get the best five results as shown in Figure 2. Through an empirical scoring function, binding affinities of the populated ligands were estimated. The screening and processing of generated molecules were done using Lipinski's rule of 5. A total of 20 unique fragment combinations were selected for further studies and the best five have been shown in Figure 2.

Table 2 Analysis of the ligands based upon the conformation, ΔG value, RMSD, and inhibition constant

S.No	Ligand Name & No.	Best Conformation No	ΔG (kcal/mol)	Ref RMSD	Inhibition Constant mM (millimolar)
1	Lig5	5	-4.98	4.85	492.10
2	Lig9	9	-3.44	3.93	422.40
3	Lig13	6	-3.98	3.55	386.14
4	LIg17	1	-4.12	3.58	410.14
5	Lig28	4	-5.96	4.90	524.28

The binding energy of grown ligands was then analyzed using MGL and Autodock tools (Panwar et al. 2021). A molecular docking experiment was done by using the Lamarckian Genetic algorithm and Local Search default parameters (Fuhrmann et al. 2010). Gibbs free energy showed significant binding between developed ligands with lower RMSD from the original conformation (Bansal et al. 2022). Activity concentration (IC50) of analyzed ligands was also found satisfactory under the micro-molar range (Table 2).

3 Results and Discussion

Structure-based drug designing approach has provided a strong platform for the researchers to perform in-silico docking and simulation studies under which they could derive *in-silico* simulation before labor-extensive wet-lab validation (Chen et al. 2012). In this study, a structure-based drug design approach was used to design a probable ligand molecule for *PTEN*. The 3D structure of the protein was modeled using ITASSER resulting in the generation of five models which were then validated using PROCHECK & PROSA. Predicted models were re-evaluated for geometry, stereochemistry checks, and energy distribution using PROCHECK. The data is in agreement with other studies reported in the literature (Wang et al. 2022). Based on the observations of validation, we have evaluated and selected the best model (Model1) with a significant C-score (80.3% core value) and goodness factor value (-0.34) (Table 1). The energy refined models of *PTEN* were generated using Iterative threading assembly refinement (ITASSER), ranked as per the cluster size. The model obtained have a higher C Score and better quality which is consistent with earlier reports in the literature as well (Zheng et al. 2021). The active site revealed HIS at 123 positions, Lysine at 125 positions, GLY at 127 positions, and LYS at 128 positions respectively. The literature search revealed that the active triad plays a crucial role in the substrate recruitment mechanism. Further seed molecules were surfed over NCBI Pub-chem. The screening and processing of generated molecules was done using Lipinski's parameters, which revealed the parametric division of the best compound having a molecular weight (MW) of 528.28, which is in agreement with earlier reports in the literature where MW fits over accepted rules of SBDD approach having many hydrogen bond donors and acceptors as HBD-3, HBA-7, with a logP value of 5.28, and the topological polar surface area (PSA) corresponds to 102.38. The molecule was reported to be the best possible ligand having distinct inhibition properties. The Genetic Algorithm under a Growing strategy of Ligbuilder (v1.2) was used with which more than 500,000 molecules populated the ligand using existing libraries in the program (Yuan et al. 2020). From the designed library of molecules, the best ten candidates have been selected for performing docking studies using Autodock which revealed Lig28 with the best -5.96 ΔG (kcal/mol) binding energy (Raghav et al. 2021). The Docking experiment was performed using the Lamarckian Genetic algorithm and Local Search default parameters with ΔG values referring to significant binding between developed ligands (Sharma et al. 2022d).

Conclusions

Despite some limitations especially with *PTEN* assessment as there are consistency and reproducibility issues with various types of assays including immunohistochemistry testing and scoring systems such as H-score, percentage of positive cells, and protein levels, still, the current in-silico study establishes the prognostic and/or predictive role of *PTEN* in breast cancer therapeutics. With many binding sites available in the *PTEN* complex, molecular modeling of the complex has been performed. Substrate recruiting tetrad was targeted with irreversible binding to arrest the substrate recruiting mechanism and hence inhibition of *PTEN* pathway. A library of developed ligands consisting of 50 potential candidates was screened for finding the best ligand having better energy and biosafety. LIG28 was found promising with enough binding affinity and the best-fitted biosafety parameters, which can act as a potential drug molecule. The study could be further extrapolated to understand the clinical utility of *PTEN*-loss in cohorts of patients. Therefore, the potential of *PTEN* as a biomarker in breast cancer is promising and deserves further investigations to establish the targeting of *PTEN* as a breast cancer therapeutic. The potent ligand molecules designed can pave the way for therapeutic implications in breast cancer especially targeting the Phosphatase and tensin homolog.

Conflict of Interest

There exists no conflict of interest amongst authors regarding the publication of this manuscript.

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