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Molecular Docking Studies of Wildtype P53 with Damnacanthal and Scopoletin-Phytochemicals Derived from Morinda Citrifolia

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Abstract

Cancer is a prominent cause of mortality globally, and it has the potential to impact any region of the body. The tumor suppressor protein P53 plays a significant role in inhibiting tumor growth by regulating DNA repair, inducing cell cycle arrest, promoting senescence, and triggering apoptosis when confronted with genotoxic stress. P53 is found mutated in 50% of cancers where it loses its tumor suppressive properties and gains oncogenic properties where it functions in a contradictory way in cancer cells when compared to normal cells. The mutated P53 forms oligomers with wtP53 rendering it incapable of carrying out its apoptotic functions and instead uses its prosurvival functions in promoting the tumor growth. Small ligand molecules that can bind the wtP53 and inhibit its oligomerisation with mutant P53 can be a possible therapy for cancer. Another possibility of targeting wtP53 in a cancer cell can be the induction of apoptotic function instead of pro-survival function which can be useful in eliminating the cancer cell whose damage is beyond repair. There are various therapeutic interventions available for cancer therapy but they are not without serious side effects which generates a need to develop drugs that do not have many side effects and that do not harm normal cells. In present times plant based phytochemicals have gained recognition for having many anticancer therapeutic properties among which Morinda citrifolia, also known as noni is found to have many phytochemicals with anticancer effects that can be exploited as potential therapy for cancer. In this study we used two PDBID structures of P53, PDBID-6FF9, containing the N-terminal domain (NTD) and the DNA binding domain (DBD) and PDBID-3SAK containing only the tetramerisation domain (TD) of wtP53 and analysed the docking of these structures with two of the phytochemicals (Damnacanthal, Scopoletin) present in Morinda citrifolia which are known to have potential anticancer therapeutic value. The two ligands were found to interact with the protein and the best docking position with the lowest binding energy was selected and reported in this paper.

Keywords: Morinda citrifolia, Damnacanthal, Scopoletin, Tumor suppressor protein P53, Phytochemicals.

INTRODUCTION

Cancer, also known as a malignant tumor or neoplasm, ranks as one of the primary causes of mortality on a global scale. It serves as a generic term encompassing a vast array of diseases that have the potential to impact any part of the body. Cancer originates from the conversion of normal cells into tumor cells through a multi-stage process, typically advancing from a pre-cancerous lesion to the development of a malignant tumor. There are three main categories of genes that play crucial roles in cancer development: oncogenes, tumor suppressor genes, and DNA repair genes. Tumor suppressor genes are responsible for

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regulating cell division and replication. The proteins produced by these genes work to restrain cell proliferation or survival. When these regulatory functions fail, a cell may undergo changes that can lead to cancerous growth [1, 2].

There are several tumor suppressor proteins such as Retinoblastoma1 (RB1), Adenomatous polyposis coli (APC), Breast cancer type1 susceptibility protein (BRCA1), Cyclin dependent kinase imhibitor2A (CDKN2A) etc. Among these P53 is a tumor suppressor protein which is vital in maintaining DNA stability and preventing cancer. A transcription factor, P53, plays a pivotal role in modulating cell cycle arrest, senescence, apoptosis, and DNA repair when exposed to genotoxic stress. P53 also exerts control over various other cellular functions, including promoting autophagy, cellular differentiation, ferroptosis, inhibiting invasion and metastasis, metabolic reprogramming, and regulating stem cell self-renewal, all of which collectively contribute to its tumor-suppressing capabilities. Normally, a negative regulator binds to wildtype P53 protein (wtP53) and keeps it inactivated or degrades it, but when DNA damage occurs in a cell, P53 gets activated and stops the cell cycle giving the cells a chance to repair, but if the damage is severe, the cells undergo apoptosis. P53 also exhibits transcription independent apoptogenesis, which contributes to its role in tumor suppression. The formation of a tetramer by P53 is essential for its tumor-suppressive activity because it functions as a transcription factor only when it binds to site-specific DNA response elements in the form of a tetramer.

P53 is found mutated in more than 50% of human cancers and 74% of these mutations are point mutations where a single amino acid is changed in the protein. The mutated P53 protein not only loses its wildtype function but gains dominant-negative activity where it increases it’s own oncogenic functions, leading to the tumor’s more aggressive behavior and drug resistance [3–7]. P53 oncogenic mutants not only oligomerise with each other but also oligomerise with wtP53 leading to the gain of oncogenic function and loss of tumor suppressive activity of wtP53.This oligomerisation assignes a significant number of effects to mutant P53 such as alteration of it’s tertiary structure, which forces a non wildtype conformation upon wtP53 rendering it incapable of carrying its function of apoptosis [8, 9]. In some cancer cells carrying wtP53, abnormal signaling of the P53 pathway occurs, where instead of triggering apoptosis, the pro-survival activities (DNA repair, cell cycle regulation) of wtP53 contributes to the overall survival potential of the cells and counteract cytotoxic effects of tumor therapies targeted towards DNA damage pathways [10]. In this case small drug ligand molecules that can bind to wtP53 preventing its oligomerisation with mutant P53 and inhibiting its pro-survival activities can be a possible therapy for cancer. P53 serves as a valuable biomarker for tracking tumor progression and represents an ideal target for developing cancer treatment strategies. This is because not only mutated P53 but also the wild-type P53 can contribute to cancer progression.

There are many therapeutic interventions available to treat cancer which majorly target rapidly dividing cells among which several rapidly dividing normal cells in our body like bone marrow cells, digestive tract cells and hair follicle cells are also affected by the drugs and as a result, serious side effects emerge. These side effects encompass myelosuppression, mucositis, hair loss, cardiotoxicity, neurotoxicity, and immunosuppression. Another limitation lies in the resistance of cancer cells to currently available drugs, often achieved through the acquisition of mutations. Considering the less-than-optimal outcomes of chemotherapy, current research is heavily focused on innovative approaches for treating cancer with reduced side effects. The perfect anticancer medication would specifically focus on and eliminate cancerous cells while preserving the health of normal ones. Based on research findings, phytochemicals and derivatives found in plants show promise as a potential option for more effective and less toxic cancer therapy. Phytochemicals, which are secondary metabolites found in numerous medicinal plants, hold substantial anticancer potential due to their diverse medicinal and nutraceutical properties. The utilization of plant-based bioactive extracts and phytomedicines is increasingly acknowledged due to their structural diversity, minimal side effects, high bioavailability, and the ability to exhibit multiple target activities [3, 11].

Morinda citrifolia also called as noni has many phytochemicals with many pharmacological activities, some of which are reported as natural anticancer cure. *M. citrifolia* has shown diverse anticancer properties in various cancer models through multiple mechanisms, encompassing antitumor effects, inhibition of proliferation, promotion of apoptosis, suppression of angiogenesis, inhibition of cell migration, anti-inflammatory actions, and immunomodulatory activities. *M. citrifolia* is considered a potentially valuable medicinal plant for cancer treatment due to its numerous inherent pathways [12, 13].

In the present study we have analysed the docking of two of the natural phytochemicals present in Morinda citrifolia which are damnacanthal and scopoletin with wtP53 to see the best docking positions involving various bonds with the amino acid residues with the lowest binding energies.

MATERIALS AND METHODS

Sequence and Structure Retrieval of Human P53

The amino acid sequence of human p53 was obtained from the Uniprot knowledgebase [14] using the search term "human p53." The first result with the UniProt ID P04637 was selected, and the amino acid sequence of human p53 was retrieved in FASTA format. Subsequently, a search for the Protein Data Bank (PDB) structure of human p53 was conducted on the RCSB website. org [15] and proteins with PDBIDs 6FF9 and 3SAK were retrieved.

Preparation of Protein for Docking Studies

The proteins were prepared for docking studies using three open softwares which are PyMol [16], Discovery Studio 2020 (BIOVIA) and Swiss PDB Viewer (SPDBV). Pymol was used to convert the mutated form of the protein to the wildtype form for 6FF9 PDB structure. Discovery Studio 2020 (BIOVIA) was used to remove water molecules and other unwanted chains and Heteroatoms for both 6FF9 and 3SAK structures. After cleaning the structures, SPDBV was used for energy minimization of the proteins and saved in PDB format for docking studies to be done in PyRx-v0.8

Structure Retrieval of LIGANDS

Four phytochemicals with anticancer properties from Morinda citrifolia were considered as ligands for present docking studies which are Damnacanthal, Citrifolinoside, Ursolic acid and Scopoletin. The structures of each of the ligand were retrieved from pubchem [17] by saving them in SDF format.

ADME Analysis of Ligands used for Docking Studies

ADME, short for Absorption, Distribution, Metabolism, and Excretion, refers to investigations aimed at understanding the processing of a chemical or drug compound within a living organism [18].

The ligands examined for ADME analysis include Damnacanthal, Citrifolinoside, Ursolic acid, and Scopoletin. Their drug-likeness was assessed using the Swiss ADME web tool [19] by inputting the canonical SMILES obtained from PubChem. The drug-likeness of these compounds was evaluated based on Lipinski's rule parameters, and their gastrointestinal absorption was assessed using the BOILED-Egg model.

Molecular Docking Studies of wtP53 with the Ligands

Molecular docking is a commonly used computational technique that predicts the most stable orientation of a ligand within the active site of a specific target by calculating the Gibbs free energy. In this context, the lowest or most negative score indicates the most stable and potentially potent complex. Molecular docking analysis was performed using the PyRx-v0.8 virtual screening tool in combination with AutoDock-Vina, utilizing the Lamarckian genetic algorithm. Energy minimization for the ligands was carried out using the MMFF (Merck Molecular Force Field) force field. A blind docking approach was adopted to predict the docking modes and binding energies. The docking was conducted with an exhaustiveness value set to 8, while all other docking parameters were maintained at their default values in the software [20].

Visualization of Protein-ligand Complex

The optimal conformation for each protein-ligand complex was generated using Discovery Studio 2020 (BIOVIA) following the docking process. The amino acid residues responsible for critical interactions that bind the ligand within the active site were determined through the 2D diagram analysis.

RESULTS

Sequence and Structure Retrieval of Human P53

The human P53 amino acid sequence was obtained from the Uniprot knowledgebase using the search term "human P53." The very first result of the cellular tumour antigen P53 of 393 amino acids length with the uniprot ID P04637 was selected and retrieved in FASTA format. P53 is coded by TP53 gene located at the chromosome17 [21]. The Protein Data Bank (PDB) structure for human P53 was searched for in the RCSB PDB database.org and a protein with PDBID 6FF9 consisting of 4 chains – A, B, C, D (tetramer) was retrieved. The structure has a sequence length of 193 amino acids of each monomer and was determined by X-ray diffraction with resolution of 2A^0. The structure has a molecular weight of 87.24KDa and has a single mutation at 280th position where arginine was mutated to lysine (R280K) (Figure 1). Another PDB structure consisting of only the oligomerisation domain of the protein P53 with PDBID 3SAK having a sequence length of 42 amino acids was retrieved. The structure has a molecular weight of 19.79KDa consisting of 4 chains with 23 conformers determined by high resolution solution NMR [22] with no mutations (Figure 2).

Structure of Human P53

The P53 monomer consists of 393 amino acids organized into five domains. These domains include an intrinsically disordered N-terminal transactivation domain (TAD) spanning amino acids 1–42, and a proline-rich domain spanning amino acids 61–92, a core domain or central site-specific DNA binding domain (DBD) comprising of amino acids 101–300, a tetramerization/oligomerisation domain (TD/OD) comprising of amino acids 326–356 and a C-terminal basic domain comprising of amino acids 364–393. In its folded form, the DBD exhibits a β-sandwich topology with two loops and a loop-sheet-helix motif. The structural zinc(II) ion is coordinated by four specific amino acid residues, namely C176 and H179 (situated within loop L2), as well as C238 and C242 (situated within loop L3). This coordination plays a crucial role in maintaining the correct folding of the DNA-binding domain (DBD) by stabilizing these two loops. The TD allows p53 monomers to ensemble as a tetramer which is crucial for its transcriptional activity. In its active conformation, P53 binds cooperatively to specific DNA sequences known as response elements (RE), which are typically located within the promoter region of target genes [4, 23].

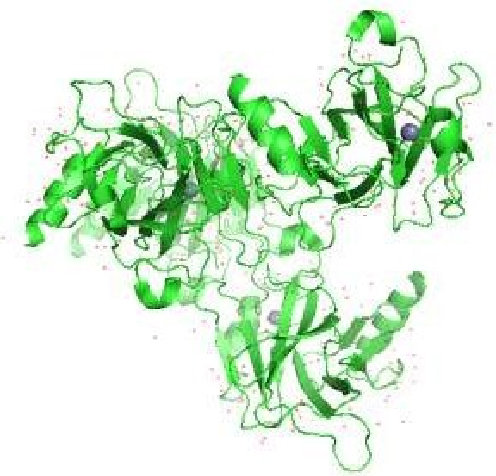

Figure 1. PDBID-6FF9. Crystal tetramer structure of mutant R280K of P53 determined by X-ray diffraction with resolution of 2A^0. The PDB structure was saved as PNG by using PyMol.

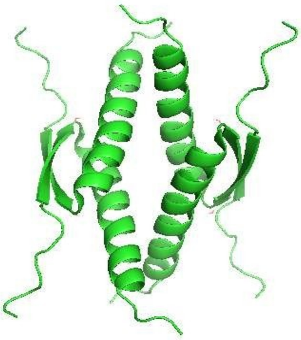

Figure 2. PDBID-3SAK. High resolution solution NMR structure of the oligomerisation domain of P53. The PDB structure was saved as PNG by using PyMol.

Preparation of Protein for Docking Studies

As our objective was to analyse the interaction between wtP53 and the ligands, we did not need the mutation in the 6FF9 structure. The mutated (R280K) P53 was converted to wtP53 in PyMol by selecting the position of mutation and applying mutagenesis wizard and changing the mutated form (Lysine at 280) to wildtype form (Arginine at 280) in chain A. This wtP53 PDB structure of the protein was loaded in Discovery Studio (BIOVIA) and the water molecules were removed along with all the chains except chain A as we did not need the tetramer conformation of the protein. The cleaned structure of P53 was loaded in Swiss PDB Viewer (SPDBV) for energy minimization and saved in PDB format for docking studies to be done in PyRx-v0.8 (Figure 3, Figure 4).

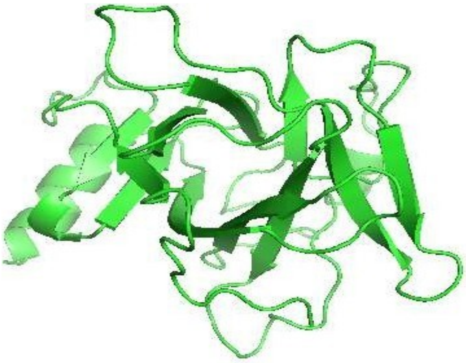

Figure 3. PDBID-6FF9. Wildtype P53 protein monomer prepared for molecular docking using PyMol, Discovery Studio (BIOVIA) and SPDBV open softwares.

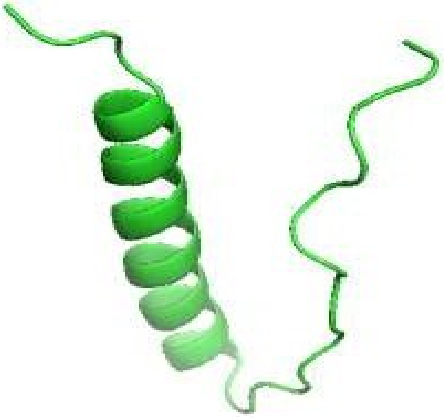

Figure 4. PDBID-3SAK. The oligomerisation domain of wtP53 monomer prepared for molecular docking using PyMol, Discovery Studio (BIOVIA) and SPDBV open softwares.

ADME Analysis of Ligands used for Docking Studies

The ligands Damnacanthal, Citrifolinoside, Ursolic acid, and Scopoletin were assessed for ADME properties, including druglikeness, using the Swiss ADME web tool. This analysis was performed by inputting the canonical SMILES representations of these ligands, which were obtained from PubChem. The druglikeness of these compounds was evaluated based on Lipinski's rule parameters, and their gastrointestinal absorption was determined using the BOILED-Egg model.

Damnacanthal ($C_6H_{10}O_5$) is an alkaloid phytochemical present in Morinda citrifolia, and it is recognized for its ability to inhibit the growth of RAS cancer cells. Its molecular weight is 282.25 g/mol. The number of hydrogen bond acceptors and donors are 5 and 1 respectively. The ligand follows Lipinski's rule as its lipophilicity is 1.84 and it exhibits high gastrointestinal (GI) absorption, but it does not permeate the blood-brain barrier.

Citrifolinoside ($C_{28}H_{30}O_{15}$), an iridois, is known to suppress UV-B induced activator protein1 (AP1) activity. The ligand has a molecular weight of 606.53 g/mol, with 15 hydrogen bond acceptors and 4 hydrogen bond donors. It was not found to follow Lipinski's rule with the lipophilicity being -0.41 and low Gastrointestinal absorption. The ligand was not found to be blood brain barrier permeant.

Ursolic acid ($C_{30}H_{48}O_3$), is a triterpenoid with anticancer activity. The ligand has a molecular weight of 456.70 g/mol, with 3 hydrogen bond acceptors and 2 hydrogen bond donors. It was found to follow Lipinski's rule with the lipophilicity being 5.93 and low Gastrointestinal absorption. The ligand was not found to be blood brain barrier permeant.

Scopoletin ($C_{10}H_8O_4$), a coumarin derivative, exhibits anti-proliferative effects. This ligand has a molecular weight of 192.17 g/mol, featuring 4 hydrogen bond acceptors and 1 hydrogen bond donor. It follows Lipinski's rule with the lipophilicity being 1.52 and high gastrointestinal absorption. It was found to be blood brain barrier permeant [13, 17].

Out of these four phytocheicals, Damnacanthal and scopoletin were found to be better choices for considering as ligands for molecukar docking studies with wtP53 (Figure 5, Figure 6).

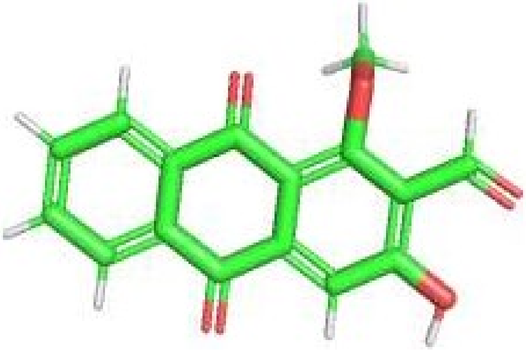

Figure 5. Damnacanthal CID-2948.

Molecular Docking Studies of wtP53 with the Ligands

The molecular docking analysis was conducted using the PyRx-v0.8 virtual screening tool in combination with AutoDock-Vina, utilizing the Lamarckian genetic algorithm. Both the ligands were individually docked with the wtP53 (6FF9) and wtP53 tetramerisation domain (3SAK) as separate docking runs. Energy minimization was carried out for ligands using mmff force field. We employed a blind docking approach to predict docking modes and assess binding energies. Blind docking has been proposed as an effective and reproducible method for molecular screening, offering relative ease of use. The grid box selection enabled the ligand to move within the defined boundaries in the X, Y, and Z planes, allowing for flexibility during the docking process. The 6FF9 grid box dimensions were set as 61.8955912945 x 53.4623490841 x 52.7063422902A^{0} centered at 73.5331466509 x 0.0893054413379 x 113.895385945. The 3SAK grid box dimensions were set as 45.9885090017 x 52.5767798312 x 52.3489827041 centered at 7.3685410406 x 11.0096942919 x 5.49160957506. The study grouped the outcomes according to the root mean square deviation (RMSD) criterion and chose ligands with modes that had an RMSD of 0. Docking was carried out with an exhaustiveness setting of 8, while all other docking parameters were maintained at their default values in the software. The ligands interacted with the protein, and the best conformation was determined based on the lowest binding energies.

Figure 6. Scopoletin CID-5280460 2D structure of Scopoletin CID-5280460 obtained from PyMol.

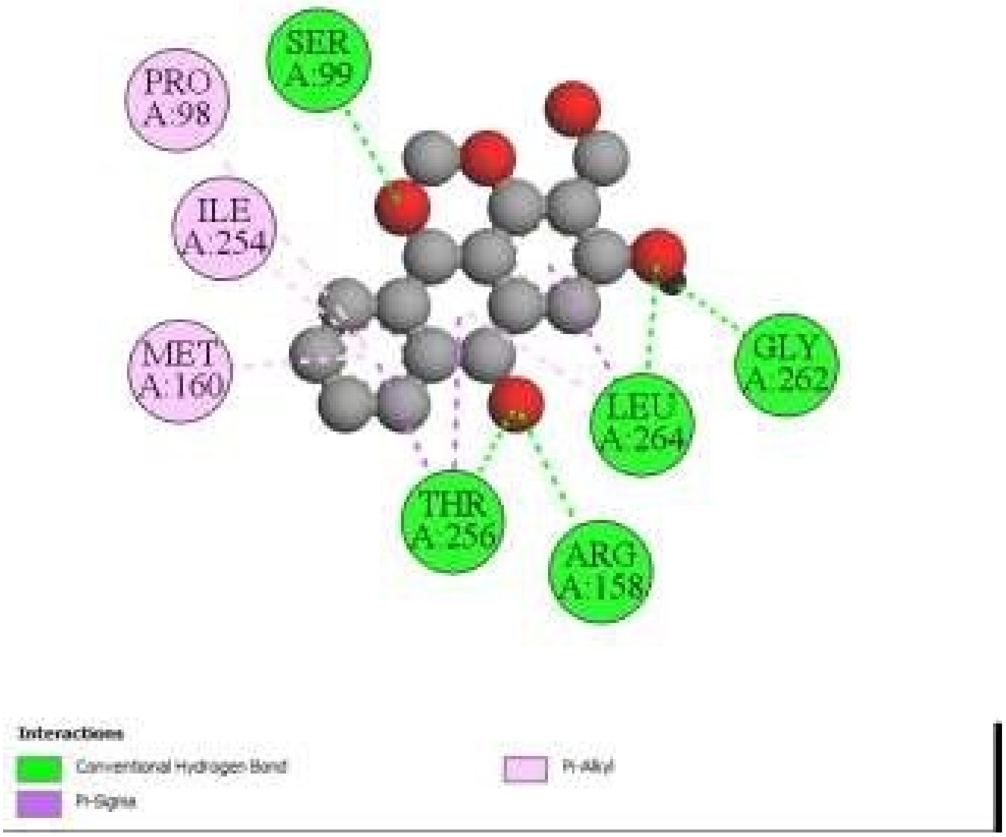

Figure 7. The ligand Damnacanthal CID2948 was found to bind with wtP53 (6FF9) with hydrogen bonds at Ser99, Arg158, Thr256, Gly262 and Leu264 and Pi-alkyl bond at Pro98, Met160 and Ile254 and Pi- sigma bond at Thr256 and Leu264.

Visualization of Protein-ligand Complex

At the conclusion of the docking process, Discovery Studio 2020 (BIOVIA) was utilized to generate the best conformation for each protein-ligand complex. The 2D diagram was used to reveal the amino acid residues engaged in critical interactions responsible for binding the ligand within the active site. The ligand Damnacanthal was found to bind with wtP53 (6FF9) with hydrogen bonds at Ser99, Arg158, Thr256, Gly262 and Leu264 and Pi- alkyl bond at Pro98, Met160 and Ile254 and Pi-sigma bond at Thr256 and Leu264 with the binding energy being -7.2 (Figure 7). The ligand Damnacanthal was found to bind with wtP53 tetramerisation domain 3SAK with hydrogen bonds at Asn27, Carbon-hydrogen bond at Glu31 and Pi-alkyl bond at Leu12 and Arg24 with the binding energy being -5.9 (Figure 8). The ligand scopoletin was found to bind with wtP53 6FF9 with hygrogen bonds at Glu258, Carbon hydrogen bond at Arg158, Pi-sigma bond at Thr256 and Leu264 and Pi-alkyl bond at Met160 and Leu264 with the binding energy being -5.5 (Figure 9). The ligand scopoletin was found to bind with wtP53 tetramerisation domain 3SAK with hygrogen bonds at Arg17, Pi-pi stacked bond at Phe20 and Pi-alkyl bond at Ile14, Arg17 and Phe20 with the binding energy being -5 (Figure 10).

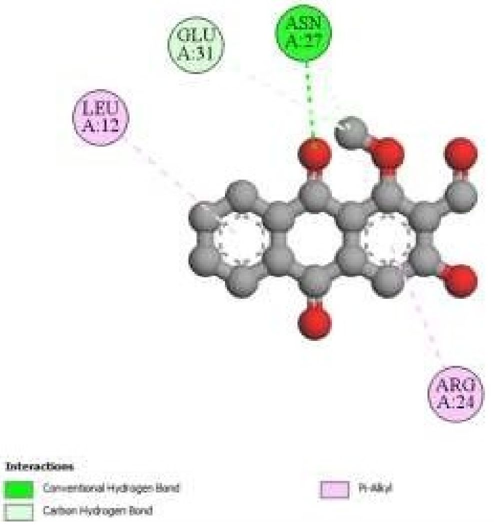

Figure 8. The ligand Damnacanthal CID2948 was found to bind with wtP53 tetramerisation domain 3SAK with hydrogen bonds at Asn27, Carbon-hydrogen bond at Glu31 and Pi-alkyl bond at Leu12 and Arg24.

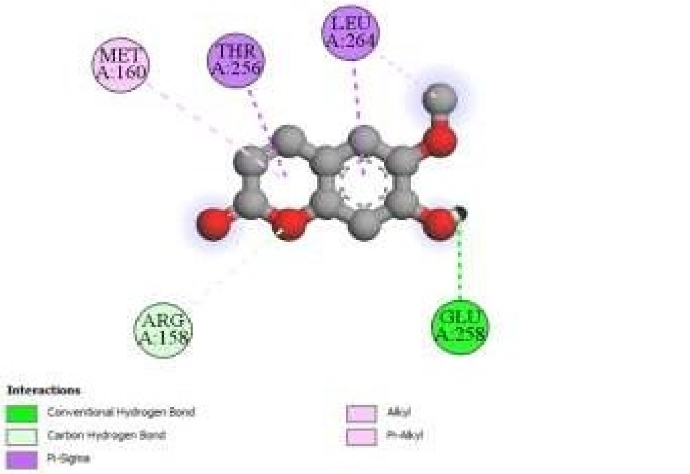

Figure 9. The ligand Scopoletin CID 5280460 was found to bind with wtP53 6FF9 with hygrogen bonds at Glu258, Carbon hydrogen bond at Arg158, Pi-sigma bond at Thr256 and Leu264 and Pi-alkyl bond at Met160 and Leu264.

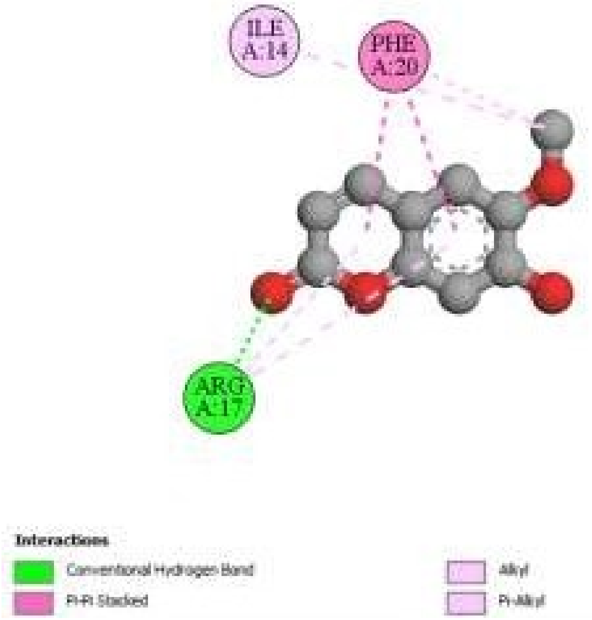

Figure 10. The ligand scopoletin CID 5280460 was found to bind with wtP53 tetramerisation domain 3SAK with hygrogen bonds at Arg17, Pi-pi stacked bond at Phe20 and Pi-alkyl bond at Ile14, Arg17 and Phe20.

DISCUSSION

The conserved tumor suppressor P53 functions as a transcription factor that reacts to stress signals. It controls apoptosis through a series of quantitative and qualitative events, which involve several binding partners [24, 25]. It has a role in many diseases [26–33] especially in various types of cancers where it's gene is the most frequently mutated one and the majority of them are missense mutations. As a result, these mutant p53 either directly lose wildtype P53 (wtP53) tumor suppressor function or apoptotic functions or exhibit a dominant negative effect over wtP53 and acquire new oncogenic functions (gain of function) [25].

The wild-type P53 effectively suppresses cell proliferation by halting cell cycle progression and encouraging apoptotic cell death. This effectively hinders tumor cell growth and inhibits cancer development. P53 can be activated by a wide array of stress signals that a cell may encounter during malignant progression. These signals encompass genotoxic damage, oncogene activation, disruption of normal cell contacts, and hypoxia, among various others. Upon activation, P53 initiates its growth inhibitory functions, which are typically dormant in normal cells. But it is not as simple as it seems. Some P53 functions do not require activation by acute stress and P53 can promote entirely contradictory outcomes in a cancer cell. If some p53 functions that normally contribute to tumor suppression are not properly regulated, they might switch sides to help promote cancer development, for example the prosurvival functions of P53, which protect cells undergoing repair following mild stress but these prosurvival functions would be extremely counterproductive if maintained in an irreparably damaged cells. A tight restriction over some p53 responses may be necessary for it's functions to be used in a productive manner which favors normal cells instead of cancer cells, suggesting that inhibition of p53 might be useful in cancer therapies [10, 34].

For P53 to carry out its transcriptional functions, it's specific DNA binding is important. It is reported that the NTD interacts with the DBD and increases the selectivity of P53 for specific DNA by improving the ability to distinguish between specific and nonspecific DNA sequences [35]. To activate P53 and enable its tumor suppressive function, tetramerization facilitated by its tetramerization domain is necessary. This activation leads to the induction of transcriptional programs that determine cell fate, including options such as cell cycle arrest or apoptotic cell death [36].

Mutant P53 in cancer cells forms oligomers with wtP53 through their tetramerisation domain and suppresses the tumor suppressive functions or apoptotic functions of endogenous wtP53 and instead uses its prosurvival functions for the cancer progression [37, 38]. Small ligand molecules that can bind the wtP53 and inhibits its oligomerisation with mutant P53 can be a possible therapy for cancer. Another possibility of targeting wtP53 in a cancer cell can be the induction of apoptotic functions instead of pro- survival functions which can be useful in eliminating the cancer cell whose damage is beyond repair.

There are various therapeutic interventions available which act on specific molecule of specific pathways [39]. But the treatments available are not without side effects. There are two ways through which drugs can be used one is by drug repurposing [40] and the other is drug development. There is a need to develop drugs without many side effects and that do not harm normal cells. In present times plant based phytochemicals have gained recognition that have many therapeutic values. Morinda citrifolia also known as noni has many phytochemicals with anticancer effects that can be exploited as potential therapy for cancer [3, 11, 12, 13].

In this study we used two PDBID structures of P53, PDBID-6FF9 containing 193 amino acids sequence length, containing the N-terminal domain (NTD) and the DNA binding domain (DBD) and the other containing 42 amino acid residues, containing only the tetramerisation domain (TD) of wtp53. We analysed the docking of these structures with two of the phytochemicals (Damnacanthal, Scopoletin) present in Morinda citrifolia which are known to have potential anticancer therapeutic value. The optimal docking position, characterized by the lowest binding energy, was chosen and documented in this paper.

CONCLUSION

In silico drug discovery methods are able to reduce the time and cost for drug discovery processes. Advancements in computational methods have enabled in silico drug discovery for practical use. Several FDA-approved drugs were developed with insilico methods. The phytochemicals Damnacanthal and Scopoletin present in Morinda citrifolia were found to interact with wtP53 mostly at its DNA binding domain and tetramerisation domain, implying its potential to modulate tetramer formation, DNA binding and transcriptional activities. As there is a need to develop anticancer drugs with less side effects, these phytochemicals can be exploited for their anticancer properties.

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