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Targeting Mtor With Natural Metabolites to Manage Cancer Pathogenesis: Molecular Docking and Physiochemical Analysis

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Abstract **Introduction:** Cancer remains a significant global concern, with substantial social, public health, and economic implications, responsible for approximately one in six deaths worldwide. The mTOR protein plays a crucial role in cell signaling pathways associated with cell proliferation and development, and its dysregulation is implicated in cancer progression. Despite the known bioactive properties of Ficus virens bark extracts and the relevance of mTOR in cancer, there is a lack of studies investigating the inhibition of mTOR by Ficus virens metabolites. This study aims to explore the molecular interactions between Ficus virens secondary metabolites and mTOR through in silico methods. **Methodology:** In this study, we conducted in silico molecular interaction analyses to investigate the potential inhibitory effects of Ficus virens secondary metabolites on mTOR. The molecular docking technique was employed to predict the binding affinities and modes of interaction between mTOR and selected Ficus virens metabolites. Additionally, molecular dynamics simulations were performed to further elucidate the stability of the protein-ligand complexes. **Results:** Our findings reveal that two naturally occurring metabolites present in the methanol-based extract of Ficus virens, Dinopol-NOP and Elaidoic acid (also known as oleic acid), exhibit notable inhibitory efficacy against mTOR. Dinopol-NOP demonstrates superior mTOR inhibitory activity compared to the substrate. These compounds hold promise as potential agents for halting cancer progression by targeting mTOR signaling pathways. **Conclusion:** The results of this study highlight the potential of Ficus virens metabolites, specifically Dinopol-NOP and Elaidoic acid, as inhibitors of mTOR in cancer therapy. The in silico molecular interaction analyses provide valuable insights into the mechanisms underlying the inhibitory effects of these compounds on mTOR. Further validation through in vitro studies investigating the suppression of mTOR enzymatic activity by the isolated compounds is warranted. This research opens avenues for the development of novel therapeutics targeting mTOR in cancer treatment.

Key Words ficus virens, molecular docking, mTOR, cancer, ADME

1. Introduction

In the twenty-first century, cancer is a significant social, public health, and economic issue. It accounts for about one in six fatalities (16.8%) as well as one in four fatalities (22.8%) from non-communicable diseases (NCDs) globally. In 2022, there were about 20 million new instances of cancer (including nonmelanoma skin cancers, or NMSCs) and 9.7 million cancer-related deaths. According to estimates, one in five men and women will have cancer at some point in their lives, and one in nine men and one in twelve women will pass away from the disease. In 2022, lung cancer accounted for about 2.5 million new cases, or one in eight cancer cases worldwide (12.4% of all cancer cases). malignancies of the female breast (11.6%), colorectum (9.6%), prostate (7.3%), and stomach (4.9%) were the next most common malignancies diagnosed worldwide. Predictions based on demographics suggest that by 2050, there would be 35 million new cases of cancer annually, a 77% rise from the 2022 figure [1].

The PI3K pathway, which is triggered by insulin, nutrition, and growth factors, includes the mTOR protein. The Akt kinase, an upstream regulator of mTOR, is involved in the PI3K pathway. Strong immunosuppressive and under experimental anticancer medication rapamycin inhibits mTOR, preventing protein synthesis and stopping the cell cycle in the G1 phase. The involvement of mTOR in cell signaling linked to the proliferation and development of cells is supported by a substantial amount of research. A phosphoinositide 3-

kinase-related protein kinase that regulates cell development in response to growth stimuli and nutrition, mammalian target of rapamycin (mTOR) is commonly dysregulated in cancer. Long-term usage of the same mTOR inhibitor can lead to drug resistance; hence, new innovative approaches to drug design should be explored in order to overcome mTOR inhibitor resistance [2].

It is thought that a variety of infectious and non-communicable diseases may be effectively treated and managed with the help of natural products and the molecules they generate [3]–[12]. Natural products, such as metabolites derived from fungi and plants, are thought to be inexpensive, safe, and offer significant bioactive potential in the fight against tumors that are resistant to several drugs [13]–[17]. The naturally occurring chemical compounds found in Ficus species, which belong to the Moraceae family, are a rich source of powerful antioxidants. These substances may be used to treat a variety of diseases linked to oxidative stress, including hepatic, neurological, and cardiovascular disorders [18]–[23]. These findings confirm the long-standing use of F. virens Aiton and highlight the plant's potential as a source of innovative drugs [20], [21], [24]. Prior studies have indicated that Ficus virens bark (FVB) extract exhibited a more inhibitory effect than other plant components against hypolipidemia, HMG-CoA reductase, and free radical scavenging [12], [21], [25], [26]. Moreover, oleic acid, gamma-caryophyllene, anazol, eugenol, diethyl phthalate, and catechol were among the potential natural chemicals that may be helpful as natural medicines that were discovered in a previous study employing GC-MS analysis [21].

Thanks to technological advancements, a number of cheminformatics approaches have been developed for quick screening and optimization of chemical entities [27]–[34]. Many Using in-silico or in-vitro methods, pharmaceutical firms begin the process of discovering new inhibitors for significant regulatory enzymes or proteins for the treatment of sickness [9], [29], [30].

Chen et.al., in 2017 reported that proanthocyanidins from Ficus virens possessed anti-breast cancer (MDA-MB-231 and MCF-7 breast cancer cells) showed that the cytotoxic effects of the proanthocyanidins against MDA-MB-231 and MCF-7 breast cancer cells were in the order of stem barks proanthocyanidins (SPAs) > leaves proanthocyanidins > fruits proanthocyanidins [35]. Aqueous extract of Panchvalkala (PVaq) formulation comprises of equal ratios of the barks from Ficus glomerata, Ficus virens, Ficus religiosa, Ficus benghalensis, and Thespesia populnea. was investigated against cervical cancer in vitro and in vivo and noticed that PVaq exhibited anticancer and immunomodulatory activities against cervical cancer cells and female mouse papilloma model [36].

Despite of several bioactive potentials of Ficus virens bark extracts and role of mTOR protein in cancer progression to the best of our understanding, there is no in-silico protein inhibition studies for Ficus virens metabolites against target proteins involved in cancer progression and malignancies. To understand the mechanistic behavior of FVB metabolites for cancer management and based on the previous information, it has been hypothesized that the mTOR inhibition might be achieved by the certain metabolites of FVB. Therefore, we illustrated the molecular interactions study of Ficus virens secondary metabolites towards mTOR through in silico study.

2. Methodology

A. Preparation of ligands and protein

The Ficus virens metabolites reported through GCMS analysis in earlier studies were used as ligand to check their inhibitory potential through computational study against structure of mTOR (PDB Id: 4JT5) in complex with native ligand (inhibitor) works as inhibitor reported by Yang, H. et al [21], [37].

B. Molecular docking and physiochemical properties

Computational screening of compounds reported earlier in Ficus virens against the target protein (mTOR; PDB Id: 4JT5) performed via PyRx-python 0.8 software (https://sourceforge.net/projects/pyrx/) based on Autodock 4.2 tool [38], [39]. The interactions was analysed with the help of Discovery Studio Visualizer ((BIOVIA Discovery Studio - BIOVIA—Dassault Systèmes®, 2021) [40]. The grid box dimensions were set to 25 x 25 x 25 Å, and centered at X: -17.80 Å, Y: -33.33, Z: -55.16. The best hits compounds of Ficus virens was analyzed through SwissADME (http://www.swissadme.ch) web-based tool [41].

3. Results

A. Molecular Docking study

In this work, fourteen compounds were chosen for molecular docking analysis, consisting of thirteen phytochemicals and one substrate, ATP, to determine the binding affinity (Kd) and binding score (delta G) for the protein of interest mTOR enzyme (PDB Id: 4JT5). Before natural chemicals were docked with proteins, the native ligand was redocked. It was observed that the redocked native ligand almost bound to the same residues as the original ligand interacted, confirming the correctness of the results (Figure 1). The native ligand that typically binds to active sites, was removed to individually dock each of the natural chemicals on the residues of the active sites.

The outcomes demonstrate that redocked native ligand resembles the reference inhibitor's binding pattern, in which native ligand co-crystallizes with the targeted protein (Figure 1A & B). The binding energies of every chemical found in the examined plant extract ranged from -4.0 to -6.3 Kcal/mol (Table 1). The binding energy of Dinopol NOP is much better than the binding energy of substrate followed by Elaidoic Acid delta G: -6.1 Kcal/mol, and Palmitic Acid and Eugenol (delta G: -6.0 Kcal/mol)

Compound Code	Compound Name	PubChem CID	δG Kcal/mol (4JT5)	Ki (M-1)
FVBM-1	Pyrocatechol	289	-4.7	$2.79x10^{4}$
FVBM-2	Palmitic Acid	985	-6	$2.50x10^{4}$
FVBM-3	Eugenol	3314	-6	$2.50x10^{4}$
FVBM-4	Quinic Acid	6508	-5.3	$7.67x10^{3}$
FVBM-5	Diethyl Phthalate	6781	-5.5	$1.07x10^{4}$
FVBM-6	Butyldiglycol	8177	-4.3	$1.42x10^{3}$
FVBM-7	Alpha-Octadecene	8217	-5.7	$1.51x10^{4}$
FVBM-8	Dinopol NOP	8346	-6.3	$4.15x10^{4}$
FVBM-9	Trimethyl phosphate	10541	-3.3	$2.62x10^{2}$
FVBM-10	Furaneol	19309	-4.5	$1.99x10^{3}$
FVBM-11	Pentadec-1-Ene	25913	-5.9	$2.11x10^{4}$
FVBM-12	Caryophyllene	26318	-5.9	$1.51x10^{4}$
FVBM-13	Docos-1-Ene	74138	-5.9	$2.11x10^{4}$
FVBM-14	2-Cyclopenten-1-One, 2-	82674	-4.3	$1.42x10^{3}$
FVBM-15	Elaidoic Acid	445639	-6.1	$2.96x10^{4}$
Substrate	Adenosine-5'-triphosphate	5957	-6.2	$3.50x10^{4}$

Table 1: Binding energy and affinity of FVBM compounds for mTOR (PDB id: 4JT5) enzyme

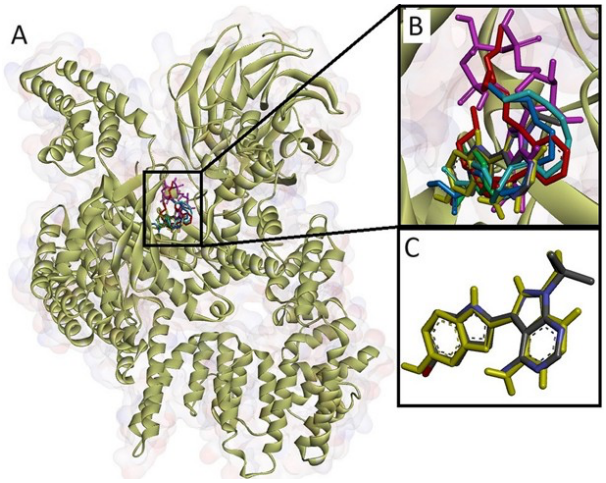

Figure 1: Molecular interaction image. (A) Superimpose image of FVBM secondary metabolites, substrate, and native ligand in mTOR enzyme, (B) Zoom in Superimpose image of best hits ligands in mTOR enzyme, (C) Zoom in Superimpose image of native ligand (grey color) and redocked native ligand (yellow color) in mTOR enzyme (RMSD: ≤ 2Å)

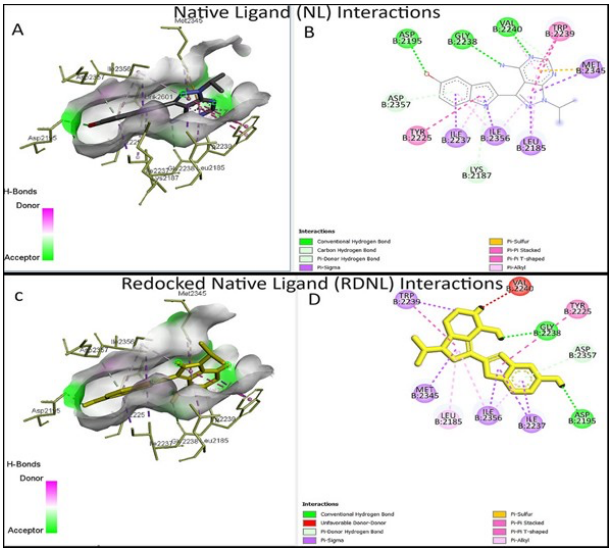

Figure 2: Interaction of target protein, (A) Hydrogen bond interactions capacity of mTOR with co-crystallized native ligand; (B) Interacting residues of mTOR with co-crystallized native ligand; (C) Hydrogen bond interactions capacity of mTOR with redocked native ligand; (D) Interacting residues of mTOR with redocked native ligand

B. Molecular interaction analysis of mTOR (PDB id: 4JT5) enzyme and native ligand (co-crystallized and redocked)

The native ligand and target protein (PDB id: 4JN5) complex stabilizes by having six hydrogen bonds with the residues VAL2240, ASP2195, GLY2238, LYS2187, VAL2240, and ASP2357; thirteen hydrophobic interactions with the residues LEU2185, ILE2237, MET2345, ILE2356, TRP2239, TYR2225, and VAL2240; two Sulphur bonds were also visible with MET2345 at distance 4.39 Å and 4.27 Å (Figure 2 A, B). Whereas the redocked native ligand and mTOR protein complex stabilizes by forming three hydrogen bonds with ASP2195, GLY2238, and ASP2357, ten hydrophobic interactions with ILE2237, MET2345, ILE2356, TRP2239, TYR2225, and LEU2185, moreover one Sulphur bond also observed with MET2345 (Figure 2 C & D).

C. Molecular interaction analysis of mTOR (PDB id: 4JT5) enzyme and FVBM compounds best hits

We found from binding energy data that Dinopol NOP and Elaidoic acid are among the best hits against the target protein so here it has been visualized the molecular interactions between ligand and target protein. The Dinopol-NOP and mTOR protein complex stabilisez with one hydrogen bond between TRP2239-Ligand, one Sulphur bond between MET2345-Ligand, and eleven hydrophobic interactions with the residue TRP2239, PRO2169, ILE2163, LEU2185, ILE2237, ILE2356, ILE2237, TYR2225, and CYS2243 (Figure 3 A,B). whereas Elaidoic acid and mTOR complex stabilizes with one conventional hydrogen bond with residue

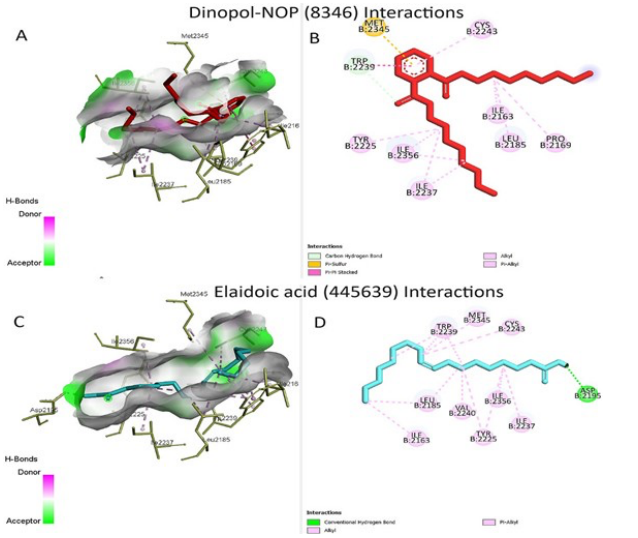

Figure 3: Interaction of target protein, (A) Hydrogen bond interactions capacity of mTOR with dinopol-NOP; (B) Interacting residues of mTOR with dinopol-NOP; (C) Hydrogen bond interactions capacity of mTOR with elaidoic acid; (D) Interacting residues of mTOR with elaidoic acid

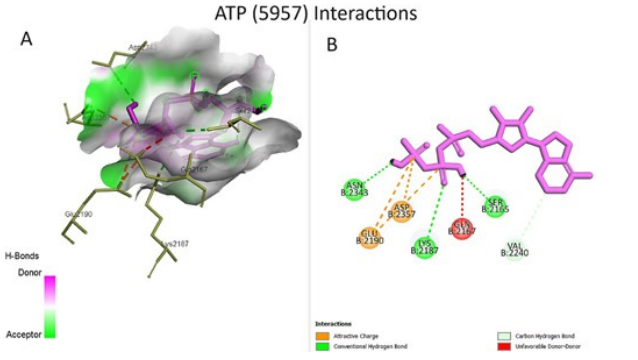

Figure 4: Interaction of target protein, mTOR with substrate ATP; (A) Hydrogen bond interactions capacity of mTOR with ATP; (B) Interacting residues of mTOR with ATP

ASP2195, and fourteen hydrophobic interactions were also observed with residues VAL2240, CYS2243, MET2345, LEU2185, ILE2237, ILE2356, ILE2163, TYR2225, and TRP2239 (Figure 3 C,D).

D. Molecular interaction analysis of mTOR (PDB id: 4JT5) enzyme and Substrate (ATP)

The substrate ATP and protein mTOR complex stabilizes by forming three Electrostatic interactions with GLU2190, and ASP2357, and five hydrogen bonds with LYS2187, SER2165, ASN2343, and VAL2240 residues (Figure 4 A,B).

E. Physicochemical properties of compounds

These physicochemical qualities are satisfied by some of the chemical compounds predicted in the methanol extract of Ficus virens that were reported as top hits (Dinopol-NOP, and Elaidoic acid) in the molecule docking investigation in this work (Table 1). According to our findings, the molecular weights of these two compounds are less than 500 KD (390.56 and 282.46 g/mol, respectively). Both the compounds have high GI absorption and cannot penetrate the blood brain barrier, while more heteroatoms, rotatable bonds, hydrogen bond acceptor were shown in Dinopol-NOP. It is acceptable that both compounds have demonstrated one instance of Lipinski rule aggression (Table 2).

Comp. Code	Dinopol-NOP	Elaidoic acid
Formula	C24H38O4	C18H34O2
MW (g/mol)	390.56	282.46
HA	28	20
AHA	6	0
FCsp3	0.67	0.83
RB	18	15
HBA	4	2
HBD	0	1
MR	116.3	89.94
TPSA	52.6	37.3
XLOGP3	8.1	7.64
GIA	High	High
BBB+	No	No
PGPs	No	No
LV	1	1

Table 2: Pharmacokinetics and physiological properties of selected four secondary metabolites (best hits) reported in FVBM extract

4. Discussion

By utilizing the computational analysis of molecular docking to identify inhibitory characteristics in various tiny molecules, the time and effort needed for wet lab labor may be reduced [42], [43]. To find the binding affinity (Ki) including binding score (delta G) for the target protein, mTOR (PDB Id: 4JT5), fourteen compounds 13 phytochemicals along with a substrate, ATP were selected for molecular docking research in this work. Elaidoic Acid & Dinopol-NOP, two components of the plant extract undergoing investigation, showed better binding affinities than any other plant metabolites this paper looked at. The protein's active site is bound by a redocked native ligand. The docking results confirm that natural substances are mTOR protein competitive inhibitors. Using molecular docking tools, the study sought to identify which metabolite (compound) could be a more effective cancer therapy. Our study supports the previous publications about Ficus virens and its anticancer properties [36]. However, there are several limitations in this work, including the absence of any analysis of RMSD, RMSF, Rg, SASA, MolSA, PSA, and other interactions that might be clarified further by studying molecular dynamics simulations. It is now well acknowledged that drug-like chemicals need to fulfill specific requirements to be successful in clinical trials as well as the drug development process. This has been verified in several previous papers [44], [45]. Four physicochemical characteristics are satisfied by 90% of orally active medicines that successfully through a phase 2 clinical

trial: molecular weight (MW) ≤ 500, log P ≤ 5, number of hydrogen bond donors (HBD)≤5, and hydrogen bond acceptor (HBA) < 10. Compounds containing more than 10 rotatable bonds often have poor oral bioavailability [46]. It is acceptable that both compounds have only demonstrated one instance of Lipinski rule aggression.

5. Conclusions

The investigation's findings indicate that two naturally produced metabolites found in the methanol-based extract of Ficus virens, Dinopol-NOP and Elaidoic acid, also known as oleic acid, had greater mTOR inhibitory efficacy, with Dinopol-NOP surpassing the substrate. These compounds could provide a better way to stop the disease from spreading. Molecular dynamics simulations and the isolated compounds' in vitro suppression of enzymes may yield requests and suggestions for further validation of this docking study.

Consent

Authors declare that this section is not applicable to this study.

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Conflict of Interest

The authors declare no conflict of interests. All authors read and approved final version of the paper.

Authors Contribution

All authors contributed equally in this paper.

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