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Exploration of natural flavonoids as novel Topoisomerase II inhibitors using DFT, molecular docking, and molecular dynamics

Tufail A.[1]*, Dubey A.[2, 3]*

[1] *Department of Biochemistry, University of Allahabad, Allahabad, India*

[2] *Computational Chemistry and Drug Discovery Division, Quanta Calculus, Kushinagar, India*

[3] *Department of Pharmacology, Saveetha Dental College and Hospital, Saveetha Institute of Medical and Technical Sciences, Chennai, Tamil Nadu, India*

** aishatufailansari@gmail.com, ameetbioinfo@gmail.com*

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Motivation and Aim: Topoisomerase II are seen as an important therapeutic target for cancer therapy now a days [1] as they are found in increased amount in cancerous cells. Topoisomerase II inhibitors are used as chemotherapeutic agents due to their ability to induce tumour cell death [2]. Role of p53 is important in controlling the cell cycle and apoptosis. As already known, p53 is unable to express in cancerous cells and is found in large amount as mutant p53. MDM2 protein is a product of one of the responsive genes of p53. As stated by the recent paper [7], the MDM2 protein, binds to the p53 protein directly through their amino termini and the function of p53 is inhibited through three major mechanisms: (1) On binding, MDM2 directly ubiquitinates p53 through its E3 ligase activity, which therefore promotes proteasomal degradation of p53; (2) p53 and MDM2 interaction blocks the p53 from binding to its targeted DNA, and hence blocking p53's transcriptional activity; and (3) export of p53 is promoted by MDM2 out of the cell nucleus, making p53 inaccessible to the targeted DNA and further its transcriptional ability is reduced. This provides a p53-MDM2 auto regulatory feedback loop. Non-Functional p53 leads to development as well as progression of tumor cells. A study shows the effect of Topoisomerase II inhibitors on the auto regulatory feedback loop of p53-MDM2 complex, which suggests an involvement of Topoisomerase II in p53 regulation [3]. This is done by activating the G1 check-point and p53-p21CIP1/WAF1 pathway [4]. One of the reasons for cancer progression is mutations in human cancer (more than 50 %) along with increased expression of MDM2 [5]. This has led to numerous evaluations and investigations in its role and potential as a therapeutic target in the sense of restoring wild type p53. As seen by the already known drugs of topoisomerase II inhibitors (e.g. doxorubicin), cytotoxic concentrations are needed for inhibiting the p53-MDM2 auto regulatory feedback loop [6]. In this study, we are targeting multiple pathway for killing cancerous cells using natural compounds. This is done by topoisomerase II inhibition along with activation of p53 by providing optimum drug concentration which would be less toxic.

Methods and Methodology: A library of natural compounds was screened *in-silico* for determining the interaction of each compound with p53-MDM2 complex (1T4F) and Topoisomerase II (4FM9) through molecular docking experiments. The already known drugs, Doxorubicin and Mitoxantrone (Topoisomerase II inhibitors) were taken as control for validation. Density Function Theory (DFT) and Electrostatic Potential

(MESP) map calculations were also performed. Following the molecular docking experiments, best protein-ligand complexes were also assessed for 100ns long molecular dynamics Simulations to record the Root means Square deviation (RMSD) between protein and ligand. RMSF were also calculated to see the variations in protein fluctuations.

Results: Each natural compound thus shortlisted, showed almost equal binding energy with both p53-MDM2 complex and Topoisomerase II (Table 1). Molecular dynamics results also shows the stability of None2 compound as compared to control Compound K (Fig. 1). Hence, indicating a possibility that same drug concentration can be given to activate p53 as needed for the inhibition of Topoisomerase II.

Table 1. Results of Molecular Docking Simulations of ligands into the active site of Topoisomerase II and p53-MDM2 complex

	Binding energy with topoisomerase II (4FM9)				Binding energy with p53+MIDM2 complex (1T4F)			
	Autodock		Glide		Autodock		Glide	
Compounds PubChem ID	best predicted binding energy, kcal/mol	number of poses in cluster with best predicted binding energy	score	Emodel	best predicted binding energy, kcal/mol	number of poses in cluster with best predicted binding energy	score	Emodel
Doxorubicin* (31703)	–6.55	2	–6.942	–77.790	–5.61	1	–3.417	–36.789
Mitoxantrone* (4214)	–5.58	1	–6.028	–62.840	–3.89	1	–4.259	–51.713
None 2	–6.02	4	–5.094	–40.652	–5.02	26	–5.867	–67.716
Acetovanillone (2214)	–4.69	2	–5.100	–32.137	–4.04	13	–4.56	–28.363
Vanillic Acid (8468)	–4.31	8	–3.995	–24.447	–4.49	21	–4.688	–23.762
Compound K (5481990)	–8.34	1			–6.18	1	–5.669	–60.991

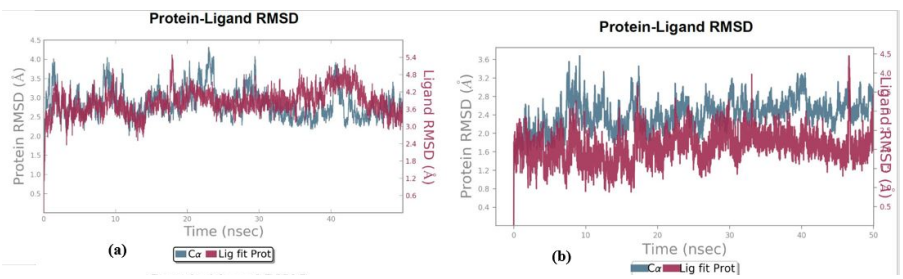

Fig. 1. Evolution of the Root mean square deviation (RMSD) during MD simulations of Topoisomerase II in complex with the 2 different ligands: (a) Compound K (b) None 2. On the left axis, the protein RMSD value (represented as the blue trace in the graph) is shown, whereas on the right axis, the ligand RMSD value (represented as the red trace in the graph) is shown. Values are reported in Å. The graphs were produced by Desmond analysis tools

Conclusion: This can probably be used as an effective therapeutic approach for curing cancers as per the strategy of targeting two pathways with the same drug concentration, unlike before where more cytotoxic concentration was needed for activating p53 apoptosis pathway than for the inhibition of Topoisomerase II. As a result, lower side effects would be seen because of drugs made of natural compounds.

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