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IN-VITRO AND THERAPEUTIC STUDY OF APIGENIN DERIVATIVE FOR THE CONTROL OF CANCER

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ABSTRACT

Studying the in-vitro and therapeutic potential of apigenin derivative for cancer control is an exciting area of research in oncology. Apigenin is a naturally occurring flavonoid found in many fruits and vegetables, known for its antioxidant and anti-inflammatory properties. Researchers have been investigating its derivative due to their potential enhanced bioavailability and efficacy in targeting cancer cells. “In-vitro and therapeutic study of apigenin derivative” discusses the use of computational techniques in drug discovery and development, specifically focusing on preparing apigenin derivative for cancer control. The study involves molecular docking analysis using autodock vina software, with selected apigenin derivative showing higher affinity as inhibitors for RALGEF-RBD compared to a reference compound. The research highlights the potential of these apigenin analogues in inhibiting RALGEF-RBD activity and emphasizes the importance of computational tools in drug discovery. Additionally, the study confirms the non-toxic nature of the synthetic compounds and suggests that these derivatives may have the potential to become effective future drugs against various diseases, including cancer.

KEYWORDS: Apigenin derivative, In-vitro, Therapeutic study, Cancer, ChemSketch software, Computational study, Novelty assessment, Drug design, Molecular docking, Target protein, 3D structure, Structure-based drug design, Ligand-based drug design, SMILES.

1. INTRODUCTION

Cancer is a condition when a many of the body’s cells grow out of control and spread to other fleshly regions. In the millions of cells that make up the mortal body, cancer and develop

virtually anywhere. Mortal cells frequently divide (via a process known as cell growth and addition) to produce New cells as the body requires them. New cells replace old bones when they die as a result of aging or damage. Sometimes, this methodical process fails, causing damaged or aberrant cells to gain when they shouldn't. Tumors, which are towel millions, can develop from these cells. Cancerous noncancerous (benign) tumors are both possible. Cancerous tumors can metastasize, which is the process by which they resettle to distant corridor of the body and foray neighboring towel to produce new tumors. Nasty tumors and another name for cancerous tumors. Malice of the blood, including leukemia, infrequently develop solid tumors although numerous other malice do. Noncancerous tumors don't access or spread to girding towel. Benign tumors generally don't come back after junking, still nasty tumors can. Still, benign tumors can sometimes grow to be extremely enormous. Some, like benign brain tumors, might have grave side effect or indeed be fatal.

1.1. Source of apigenin

Chamomile is a medicinal herb that contains a flavonoid called apigenin. Apigenin is abundant in fruits, vegetables, and seasonings such as parsley, oregano, basil, and tarragon. Many illnesses and infections, including diabetes, dysentery, hepatitis, blennorrhagia, cancer, arthritis, inflammation, woods, haemorrhoids, and leishmanial ulcers, are treated using herbs that contain apigenin.[1]

1.2. Anticancer effects of apigenin

In general, due to the source of apigenin, it appears as one of the bioactive composites of factory origin, which reduces the prevalence of cancer. Increased consumption of flavonoids from fruits and vegetables has been shown to be negatively identified with the threat of cancer. The relationship between flavonoid input (quercetin, kaempferol, myricetin, luteolin, and apigenin) was examined by Knekt etal. as well as lung cancer. Strong substantiation of flavonoids' preventative effect against lung cancer is also handed by their finding of an inverse relationship between the prevalence of cancer at all spots and flavonoid consumption. The authors came to the conclusion that apples and onions, which are rich in apigenin, parade a defensive effect against lung cancer. How salutary flavonoids are linked to their defensive among other effects, the part of cancer threat reduction were examined in the exploration done on bone cancer, ovarian cancer, and the chance of neoplasia rush following colorectal surgery individualities with cancer.[2]

2. Experimental work

2.1. Computational study

2.1.1. Design of apigenin derivative

On the basis of the availability of various functional groups such as methyl group, chlorine group, amino group, nitro group, etc. we designed 20 apigenin derivative in this project work, which summarises recent methodological advancements in the design and synthesis of new apigenin derivatives. Using the ChemSketch software, all derivative sketches produce smile notation and compute all of the attributes of the corresponding structure.[3] Continue with the computational analysis, which includes novelty assessment, toxicity prediction, molecular docking to choose and synthesise the best and most active apigenin derivative.

2.1.2. Novelty assessment of designed apigenin derivatives

We found the novelty of all 35 molecules by exploring multiple datasets such as PubChem (Compounds, 111 million entries), zinc20 (currently has 727 842 compounds), which all contains a huge dataset of accessible molecules. After searching every database, we discovered 20 unique variants that were not present in any of the databases.

2.1.3. Toxicity prediction

Acute oral toxicity prediction

To prognosticate the toxin of the input emulsion, a 2D similarity hunt is performed on an streamlined interpretation of the in- house toxin database Super Toxic (17) and the most analogous composites to the input patch are considered. The set used for vaticination consists of roughly 38 000 unique composites with known oral LD50 values measured in rodents. The data was gathered from public sources and literature and prepared using Instant JChem6.2.0 (January 2014), Chem Axon (http//www.chemaxon.com), for standardization purposes. From the standardized patch structures, InChI keys were calculated and used to remove duplicates in the dataset. In the case of multiple LD50 values measured for one emulsion, the smallest cure value was kept to represent the worst- case toxin of a emulsion. Six toxin classes were defined grounded on the GHS bracket scheme using the LD50 thresholds of 5, 50, 300, 2000 and 5000 mg/ kg body weight. Each emulsion of the dataset was represented using a concatenated point conforming of the ‘FP2’ and ‘FP4’ fingerprints of Mychem (http//mychem.sourceforge.net/) as well as the ECFP4 point (18). The fingerprints were calculated using Open Babel (19) and JChem6.1.3 (November 2013), Chem Axon (http//www.chemaxon.com), independently. The similarity between two composites was calculated using the Tanimoto Index. In addition to the

similarity hunt, the vaticination system takes into account the presence of poisonous fractions. All composites in the database were disintegrated using RECAP (20) as well as the in- house system ROTBONDS (21). To determine fractions over represented in the most poisonous classes, a propensity analysis (22) was performed. Propensity scores (PS) were calculated for every scrap and toxin class. poisonous fractions were defined as those showing a PS above a threshold of 3 in classes I, II or III, and a PS below 1 in classes IV – VI. Grounded on these conditions, a total number of 1591 and 1580 fractions specific to toxin classes I – III, generated with the ROTBONDS and RECAP fragmentation system, independently, were contemplated for vaticination.[4]

2.1.4. Molecular docking studies

The molecular docking technique offers an appealing platform for understanding drug-protein interactions, which is helpful in drug development. In this pre-programmed experiment, a tiny molecule is introduced non-covalently into the target's binding site at a specific location of the protein. In this project, molecular docking investigations with vina wizard were used to determine the mechanism of interaction between produced apigenin derivative chemicals and the RALGEF-RBD receptor, as well as the structural properties of the protein's active region. Molecular docking performed using PyRx and discovery studio visualizer to further predict the biological activity of RALGEF-RBD and their interaction with target.[5,6] PyRx used for virtual screening and also used for multiple ligands docking in our protein of interest. PyRx provide the best facility. PyRx also has an open babel which has better an easy conversion of ligand sdf format into pdb format and there is no need to convert each and every ligand into sdf to pdb format.

2.1.4.1. Platform for molecular docking

The AutoDock vina software was used to investigate the computational docking of 8 apigenin derivative (ligand) for the RALGEF-RBD (PDB ID: 2RGF), and PyRx software was utilized for comparative docking software used for visualization.[7]

2.1.4.2. Preparation of proteins

RBD of RAL Guanosine nucleotide exchange factor [protein] were taken from protein data bank. Protein structure refined by removing water of crystallization and saved as pdbpt format for molecular docking.

2.1.4.3. Ligand preparation

For molecular docking, we employed the 2D chemical structure of all ligands. OpenBabel in PyRx software was used to 3D optimize the geometry using the UFF force field with default dynamics. All ligand structure were minimized to pdb format, which was then transformed to pdbqt format for all autodock ligands. These confirmations were used to begin molecular docking. Because the PDB, partial charge (Q), and Atom type (T) (PDBQT) formats can all be used as input in the AutoDock vina software, Open Babel was used to convert these SDF files to PDB. All molecules were chosen to target the RALGEF-RBD.

2.1.4.4. Determining compound active sites

The active sites were defined as the ligand coordinates in the original target protein grids, and the calculated Biovia Discovery Studio 4.5 was used to identify these active binding sites in the target protein. The grid box and docking evaluation findings were evaluated using the amino acids in the active sites. The protein-ligand interaction profiler (PLIP) web server and PyMol software (version 1.7.4) were used to characterize the interactions between the ligand-protein complexes with the lowest binding score.[8]

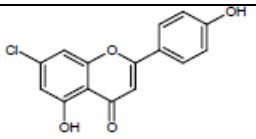
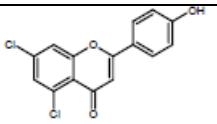
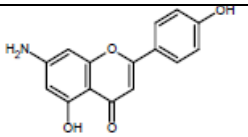
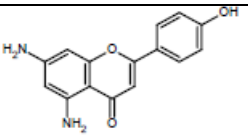
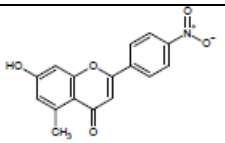
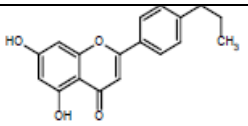
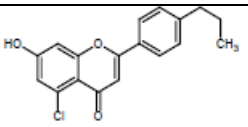
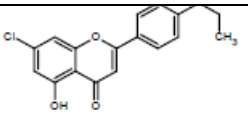
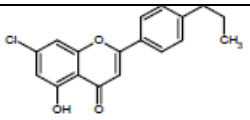
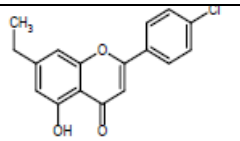
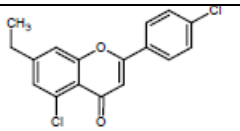
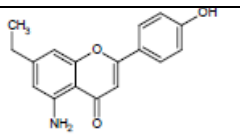
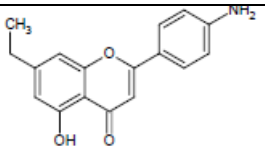
2.1.4.5. Protein ligand Docking and Visualization

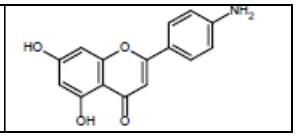
All docking experiments were conducted with AutoDock vina, with the optimized model serving as the docking target. The goal of computational docking is to develop a population of promising ligand orientations and conformations within the binding site.[9]

In molecular docking, apigenin derivative were analysed separately, and before their first interactions, the UFF force field used to optimize the structure of these small molecules, ensuring that their active sites were stiff after the docking process has been validated, the partner proteins' active sites were rigidly docked for virtual screening. Both the macromolecule and the ligand were kept inflexible throughout the virtual screening.

Finally, the binding energy limits in the software were removed, allowing the analysis of 2D hydrogen-bond interactions to be completed with the Biovia Discovery Studio 4.5 programme.[5]

Table 3: Derivative of apigenin.

ID	Smiles	Structure
SK1	O=C1C=C(Oc2cc(Cl)cc(O)c21)c1ccc(O)cc1	
SK2	O=C1C=C(Oc2cc(Cl)cc(Cl)c21)c1ccc(O)cc1	
SK3	O=C1C=C(Oc2cc(N)cc(O)c21)c1ccc(O)cc1	
SK4	O=C1C=C(Oc2cc(N)cc(N)c21)c1ccc(O)cc1	
SK5	O=[N+]([O-])c1ccc(cc1)C1=CC(=O)c2c(C)cc(O)cc2O1	
SK6	O=C1C=C(Oc2cc(O)cc(O)c21)c1ccc(cc1)CCC	
SK7	O=C1C=C(Oc2cc(O)cc(Cl)c21)c1ccc(cc1)CCC	
SK8	O=C1C=C(Oc2cc(Cl)cc(O)c21)c1ccc(cc1)CCC	
SK9	O=C1C=C(Oc2cc(N)cc(O)c21)c1ccc(cc1)CCC	
SK10	O=C1C=C(Oc2cc(cc(O)c21)CC)c1ccc(Cl)cc1	
SK11	O=C1C=C(Oc2cc(cc(Cl)c21)CC)c1ccc(Cl)cc1	
SK12	O=C1C=C(Oc2cc(cc(N)c21)CC)c1ccc(O)cc1	
SK13	O=C1C=C(Oc2cc(cc(O)c21)CC)c1ccc(N)cc1	

SK14	O=C1C=C(Oc2cc(O)cc(O)c21)c1ccc(N)cc1	

3. RESULT AND DISCUSSION

3.1. Computational study

3.1.1. Toxicity study

3.1.1.1. Protox ii

ProTox-II Webserver estimate the toxicity of compound. The toxicity parameters predicted by applying protox-II are acute toxicity, organ toxicity, toxicological endpoints, toxicological pathways, and target toxicity. This application uses molecular similarity, fragment propensity, approaches. Predictive models were developed from in vitro and in vivo experimental data set verified the model's excellent performance.[10]

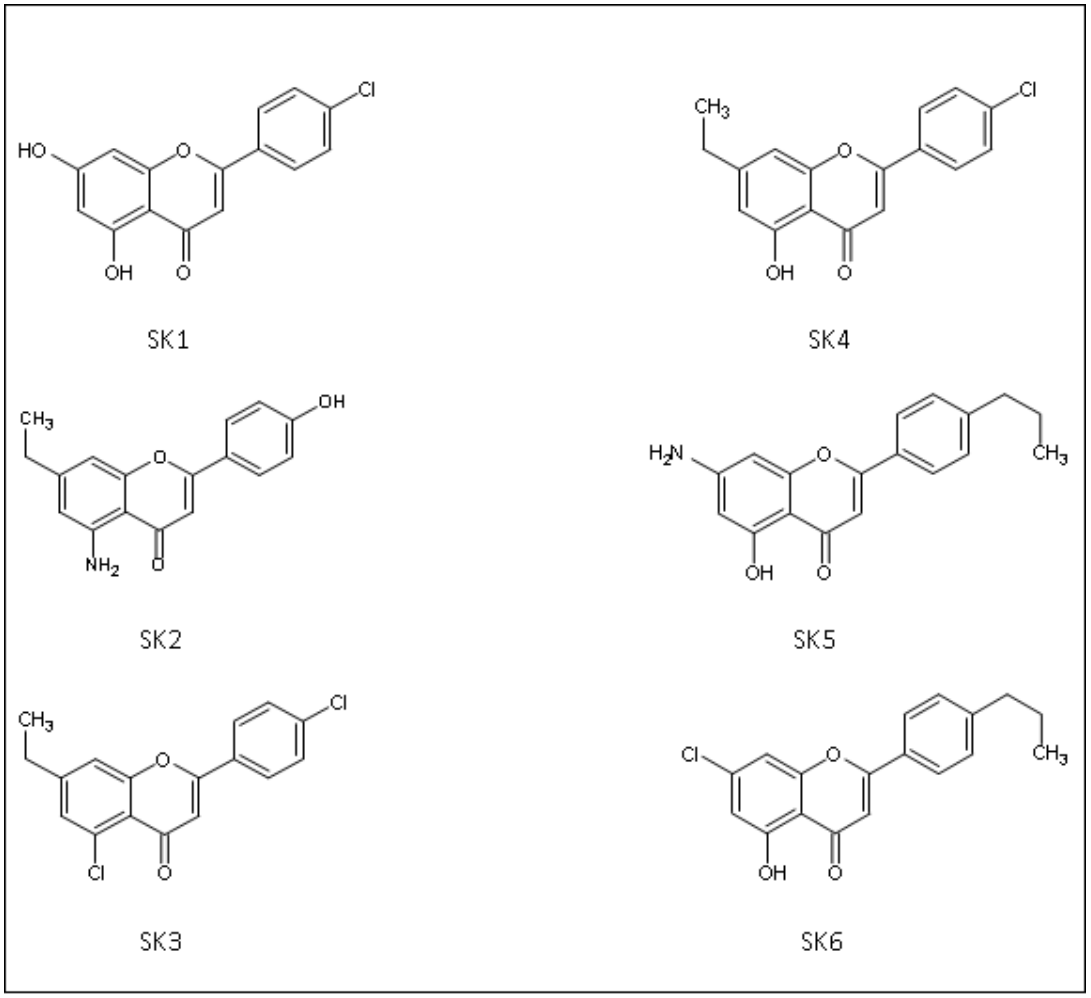


Figure 5: Selected derivative for toxicity.

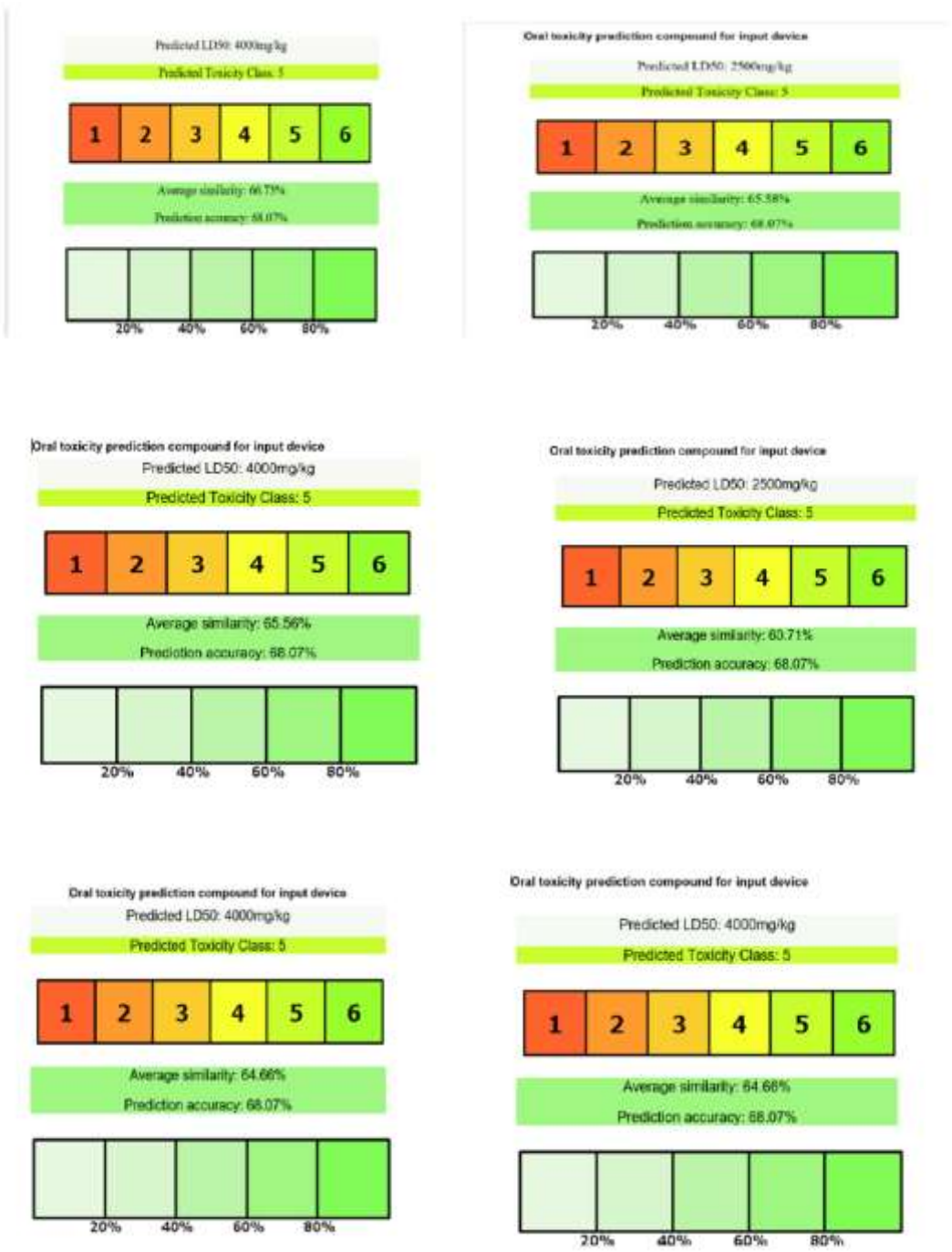


Figure 6: Toxicity Result Prediction for Selected 6 Most Activated Apigenin Against Cancerous Acitivity.

3.1.2. Molecular docking

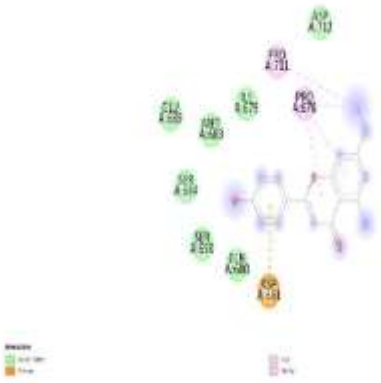
The AD Screening of 15 novel apigenin analogues assisted the discovery of 6 potential leads with the ability to inhibit RALGEF-RBD. We used autodock vina software to perform molecular docking on those novels 6 apigenin analogues.

Table 4: Ligand protein binding energy calculation for selected molecules, with ID and docking score.

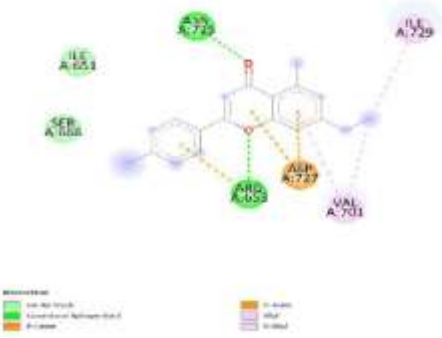
Sr. No	Derivative	Molecular ID	Docking score (kcal)
1.	2-(4-aminophenyl)-7-ethyl-5-hydroxy-4*H*-1-benzopyran-4-one	SK1	-5.6
2.	5-amino-7-ethyl-2-(4-hydroxyphenyl)-4*H*-1-benzopyran- 4-one	SK2	-5.9
3.	5-chloro-2-(4-chlorophenyl)-7- ethyl-4*H*-1-benzopyran-4-one	SK3	-5.6
4.	2-(4-chlorophenyl)-7-ethyl-5-hydroxy-4*H*-1-benzopyran-4-one	SK4	-5.6
5.	7-chloro-5-hydroxy-2-(4-propylphenyl)-4*H*-1-benzopyran-4-one	SK6	-5.6
6.	5-chloro-2-(4-hydroxyphenyl)-7-nitro-4*H*-1-benzopyran-4-one	SK7	-6.1

3.1.3. Visualization

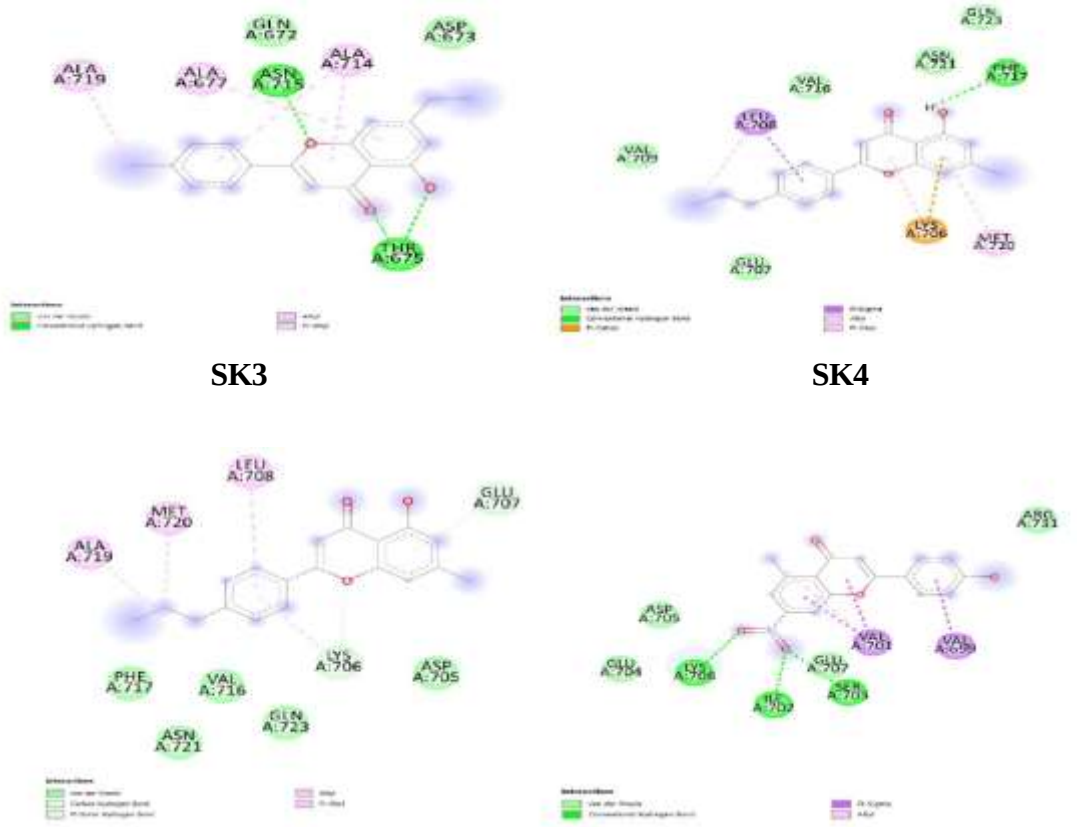
Due to its efficiency in predicting the potency of a molecule towards the reaction energy associated with potential binding conformations of an active site of biological species; evaluation of target enzyme for evaluating the binding convergence at its catalytic site, docking experiments are the most commonly used to design drugs. The docking scores of compounds SK1, SK2, SK3, SK4, SK5, SK6 were -5.6, -5.9, -5.6, -5.6, -5.6, -6.1, respectively, indicating that all 6 compounds may have substantial RALGEF-RBD inhibition action.



SK1



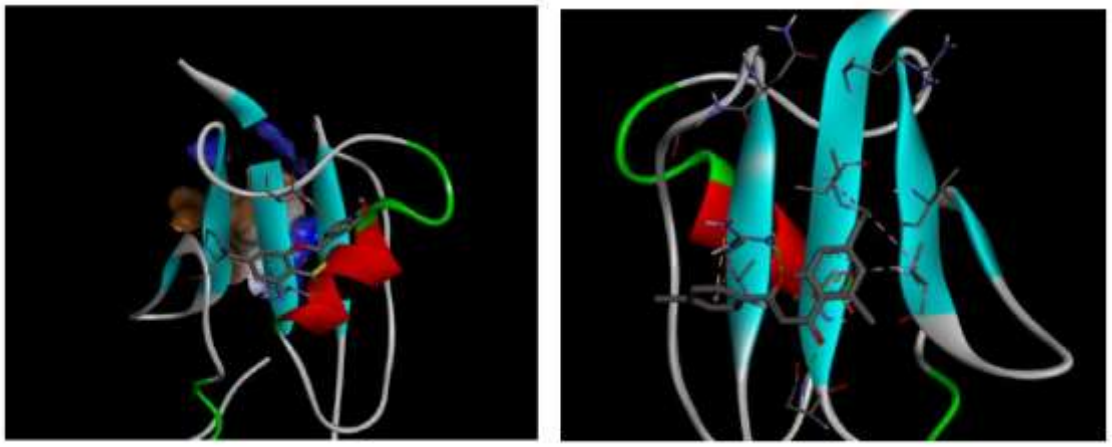
SK2



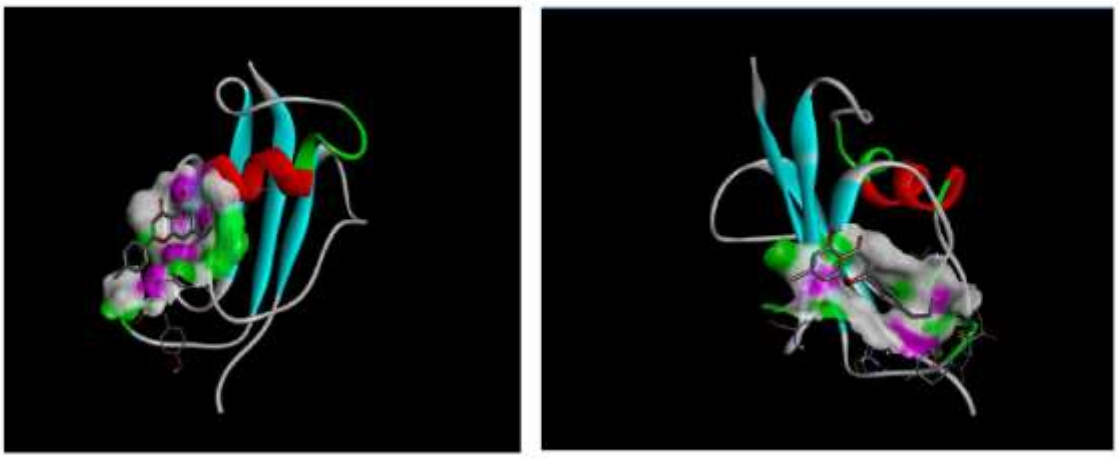
SK3 **SK4**

SK5 **SK6**

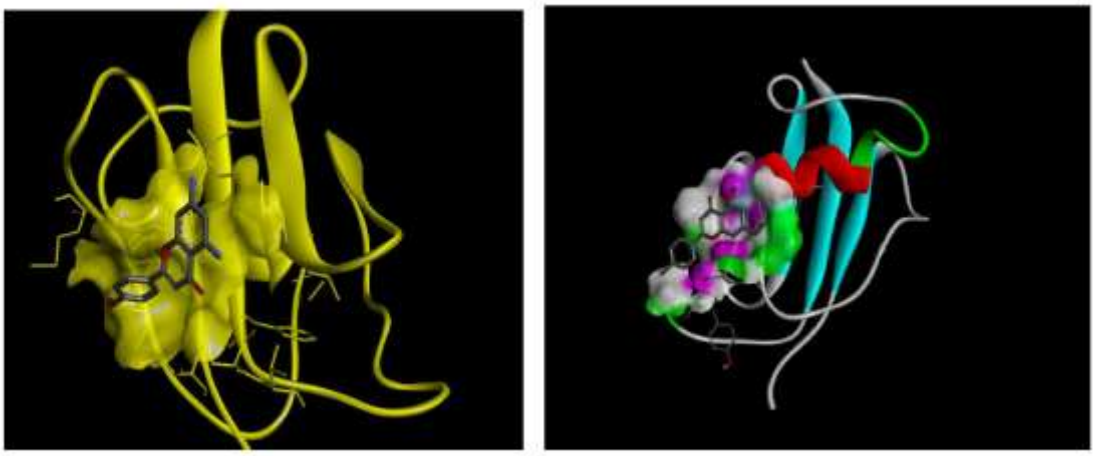
Figure 7: 2D Visualization of Active Six Molecule Which Docking Score is Higher.



SK1 **SK2**



SK3 **SK4**



SK5 **SK6**

Figure 8: 3D Visualization of Active Six Molecule Which Docking Score is Higher.

4. CONCLUSION

The study of the apigenin derivative shows promising potential for the control of cancer. Through in-vitro experiments, therapeutic studies, computational analysis, and molecular docking, researchers have identified apigenin derivative that exhibit strong inhibitory action against cancer-related proteins. The use of computational techniques, such as ChemSketch and molecular docking software, has facilitated the design and evaluation of novel molecule for cancer therapy. The findings suggest that these apigenin analogues have the ability to inhibit cancer-related proteins effectively, paving the way for the development of future drugs for cancer treatment.

5. REFERENCES

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