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In-silico APPROACH FOR VIRTUAL SCREENING AND MOLECULAR DOCKING OF FLAVONOIDS AS ERBB4 KINASE INHIBITORS IN THE TREATMENT OF CANCER

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ABSTRACT

The ongoing anticancer treatment is resulting in resistance to treatments, less efficacy, side effects, and other issues that need to be overcome by new anticancer drugs. Focusing on novel anticancer target ErbB4 kinase, responsible for tumor growth can be a promising approach against cancer. Naturally occurring plant-derived compounds are an important source of biologically active molecules with different chemical components having very little toxicity and high efficacy. This research highlights binding affinity, drug-like properties, bioactivity and toxicity prediction, and *in-silico* study of various flavonoids in cancer with the help of different software. The selected flavonoids had the lowest binding affinity which is the highest docking score, stipulating that they are efficacious and potent anticancer agents. These compounds are good contenders for further experimental investigations because of their potential for bioactivity and potential drug-like qualities.

Keywords: ErbB4, Anticancer, Virtual Screening, *in-silico*, Docking.

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INTRODUCTION

Cancer is the second leading cause of death globally. By 2040, the global cancer burden is expected to increase by 47% from 2020 and can be around 29.5 million cases in a year. The number of deaths due to cancer will also be increased to 16.4 million. Thus, there is a need to continuously research for better treatment against cancer. Cancer is mainly caused by uncontrolled function of kinases which is responsible for cellular proliferation and irregular growth. Once the kinases undergo mutation there is uncontrollable cell growth and thus inhibition of these kinases will lead to cell apoptosis and thus kinase inhibitors can be promising anti-cancer agents.[1] The epidermal growth factor receptor also known as EGFR, a family of receptor tyrosine kinases plays an essential role in cancer. HER4/ErbB4 is one of its members that is important for the normal development of vital organs and systems of the animal body like the heart, mammary gland, and nervous system. Phosphorylation of ErbB4 kinase promotes the proliferation of cancer cells by activation of RAS-MAPK-ERK & PI3K/Akt signaling pathway. ErbB4 inhibitors inhibit tumor growth and proliferation and, thus can be used for the treatment of cancer.[2] Effective anti-cancer drugs are not necessarily the result of novel drug design. Another approach to developing novel ErbB4 kinase inhibitors is the empirical screening of natural drugs available on the database for anti-cancer activity. Flavonoid is a class of natural compounds used for years as their active constituents are beneficial to humans for the treatment of various diseases. Flavonoids are a diverse group of polyphenols that are been researched for their potential health benefits, including their role in cancer prevention and treatment. These classes of compounds interfere with the cell cycle and inhibit the proliferation of tumor cells.[3]

EXPERIMENTAL

ErbB4 kinase was the principal protein target selected for the research activity. A protein's three-dimensional structure was retrieved from a protein data bank with PDB ID: 3BBT. Protein structure was downloaded in .pdb format and optimized using Discovery Studio, MGL tools, and AutoDock Vina 1.5.7.[4]

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Dataset Preparation

The database used for ligand and dataset preparation was taken from the naturally occurring Plant-based Anticancerous Compound-Activity-Target Database (NPACT). The reported flavonoids were used as ligands based on their potential anticancer properties. Information is provided about the database's in vivo and in vitro activity, SMILES and SMART notation, structure, and drug-likeness. ChemDraw and Chem3D were used for the 3D structure of ligands and their energy minimization.[5]

Drug-likeness Evaluation

ADMET properties and bioactivity prediction were tested using web servers like SwissADME, Molinspiration Cheminformatics, and ProTox II. Lipinski's rule of 5 states that the molecular weight of a compound must be less than 500 Da, the average logP must be less than 5, the number of rotatable bonds ≤ 5, no. of hydrogen bond donor ≤ 5, and no. of hydrogen bond acceptor ≤ 10. Lipinski's rule of 5 was tested using SwissADME, while bioactivity prediction was done by Molinspiration Cheminformatics. The toxicity of ligands and standard drugs was compared using ProTox II.[6,7,8,9]

General Procedure

The protein (3BBT) was downloaded from the protein data bank and saved in .pdb format. There was the removal of molecules of water and the conformation of inbuilt ligand was copied. The inbuilt ligand Lapatinib was deleted and further, the file was dragged into AutoDock Vina. The polar hydrogen was added, Kollman charges were added, and missing atoms were fixed. Finally, the protein is saved in .pdbqt file format. From the NPACT database, 329 reported flavonoid compounds were drawn using ChemDraw, and a 3D structure was created using Chem3D. An energy reduction gradient of 0.00001 kcal/mol/A was enforced for energy minimization under the force field of MMFF94. Further, these structures were subject to AutoDock for the conversion of files into .pdbqt format. Then, all the 329 ligands along with Lapatinib were subjected to docking into the binding site of an enzyme. For each ligand, 9 conformations were formed. The docking score representing the binding affinity of each conformation was predicted using the AutoDock Vina instructions. The results obtained from AutoDock Vina in the form of an output file were used to check the interactions using Discovery Studio Visualizer. The interactions of ligands and amino acids of the protein chain were compared with that of inbuilt standard Lapatinib. The compounds showing better docking scores that have less binding affinity than the standard drug Lapatinib were selected for further ADMET evaluation and bioactivity prediction.

RESULTS AND DISCUSSION

***In-Silico* Docking Results**

Cancer is one of the deadliest diseases worldwide. There have been many US-FDA drugs approved for the treatment of cancer. These approved drugs have many side effects like lymphedema, headache, blood clots, reproductive problems, loss of memory, and other symptoms. To minimize chemotherapy-induced toxins, naturally occurring compounds are thought to be promising antitumor agents. Flavonoids being the plant-derived class of compounds are taken into consideration for combating cancer by specifically targeting ErbB4 kinase. The structure of ErbB4 in combination with Lapatinib (PDB ID: 3BBT) based on X-ray crystallography was selected and all the reported flavonoids were docked against it.[10]

Validation of Docking Protocol

The AutoDock Vina docking procedure was thoroughly validated before the database's virtual screening of flavonoids. Co-crystallized ligand Lapatinib was deleted and docked back into the same binding area of 3BBT for validation and then Root Mean Square Deviation (RMSD) was checked.[11]

Structure-Based Virtual Screening

Following the validation of the docking procedure, structure-based virtual screening of the selected database was performed. The binding affinities of 329 compounds were compared with that of Lapatinib which was found to be -10.6 kcal/mol. The ranking was done based on their docking score (kcal/mol). The top 5 molecules (Fig.-1) with a binding affinity less than -10.6 kcal/mol were taken for further evaluation.

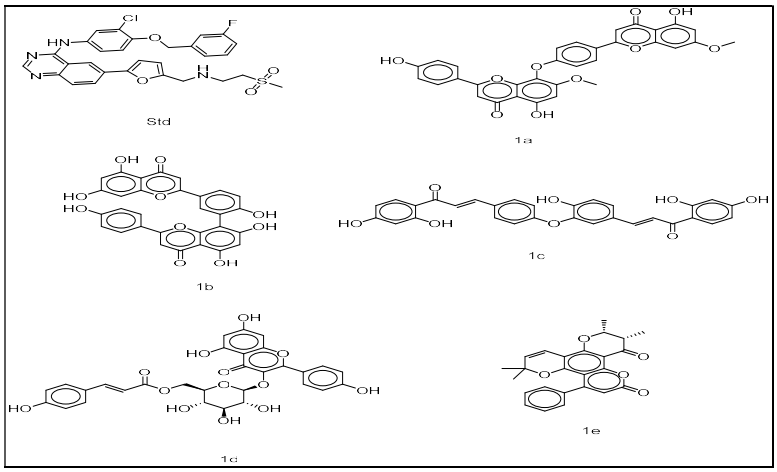

Fig.-1: Structures of 5 Best Flavonoids and Standard Drugs from Virtual Screening of Phytochemicals

Inophyllum E was found to be the best inhibiting agent with a docking score of -11.4 kcal/mol. Table-1 lists the computed values for binding affinities of standard drugs and 5 best-selected flavonoids. Further, the interactions of these compounds were checked with the protein ErbB4 kinase. Interactions necessary for the inhibition of this protein are Leu B: 699, Val B: 707, and Ala B: 724. Figure-2 shows the two-dimensional binding interaction graphs of standard drugs and the top 5 compounds.

Table-1: Compounds with Their Docking Score and Interaction with Amino Acids of Protein Target

S. No.	Name	Docking score (kcal/mol)	Amino acid residue interaction
*	Std	-10.6	Leu B:699, Val B:707, Ala B:724, Thr B:771, Asp B:818, Leu B:825, Thr B:835, Asp B:836
1.	1a	-10.7	Leu B:699, Val B:707, Lys B:726, Cys B:778, Leu B:825, Asp B:836
2.	1b	-10.8	Leu B:699, Gly B:702, Val B:707, Lys B:726, Glu B:781, Asp B:818, Leu B:825, Asp B:836
3.	1c	-11.2	Leu B:699, Gly B:702, Val B:707, Ala B:724, Thr B:771, Asp B:818, Leu B:825, Asp B:836, Phe B:837
4.	1d	-10.7	Leu B:699, Ala B:724, Met B:747, Leu B:769, Gln B:772, Cys B:778, Leu B:825
5.	1e	-11.4	Leu B:699, Val B:707, Ala B:724, Thr B:771, Asp B:818, Leu B:825, Thr B:835, Asp B:836

Std: Lapatinib; 1a: 7,7”-dimethyllanaraflavone; 1b: Amentoflavone; 1c: Luxenchalcone; 1d: Tiliroside; 1e: Inophyllum E.

ADME Evaluation and Bioactivity Prediction

Flavonoids showing better inhibitory activity based on their binding affinity were checked for Lipinski’s rule of 5. Table-2 displays all the 5 properties of selected compounds. The bioactivity score of flavonoids was checked in terms of Ion Channel Modulator (ICM), Enzyme Inhibitor (EI), G-Protein Coupled Receptor (GPCR), Protease Inhibitor (PI), Kinase Inhibitor (KI), and Nuclear Receptor (NR). Bioactivity score > 0 is active, -5.0 to 0 is less active, and < -5.0 is nonactive. Table-3 displays the bioactivity score of selected ligands.

Table-2: Properties of Standard and Selected Compounds (Lipinski rule of 5)

S. No.	Name	MW	logP	nON	nOHNH	nrotb
*	Std	581.07	6.16	8	2	11
1.	1a	566.52	6.03	10	3	6
2.	1b	538.46	5.16	10	6	3
3.	1c	510.50	5.79	8	5	8
4.	1d	596.54	1.86	13	7	8
5.	1e	402.45	5.32	5	0	1

MW: Molecular weight; logP: Partition coefficient; nON: No. of hydrogen accepting bonds; nOHNH: No. of hydrogen donating bonds; nrotb: No. of rotatable bonds.

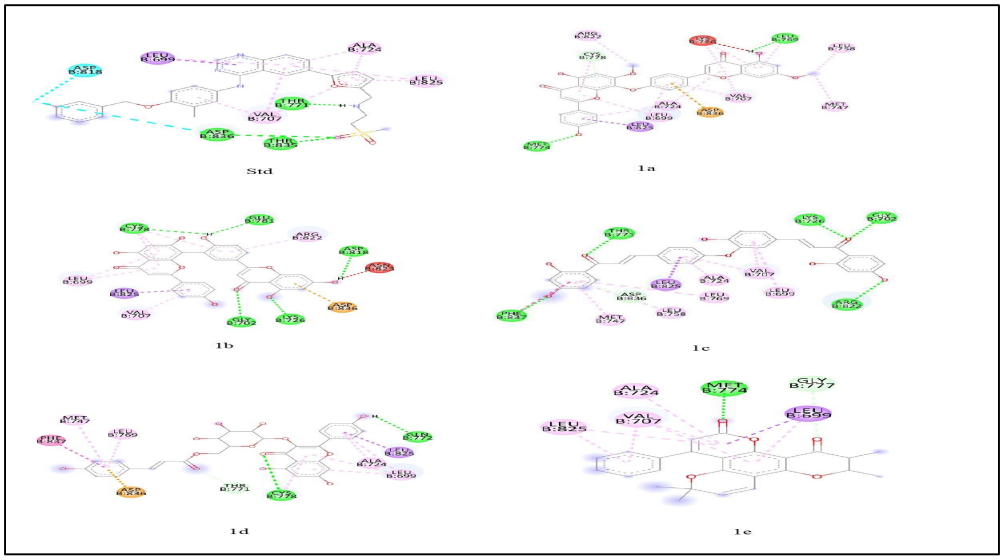

Fig.-2: Interactions of Standard and Top 5 Compounds with Amino Acids of ErbB4 Kinase

Table-3: Bioactivity Prediction of Standard and Selected Compounds

S. No.	Name	ICM	EI	GPCR	PI	KI	NR
*	Std	-0.52	-0.08	-0.04	-0.21	0.36	-0.35
1.	1a	-0.27	0.13	-0.10	-0.03	0.04	0.10
2.	1b	-0.15	0.10	0.07	0.06	0.19	0.21
3.	1c	-0.16	0.08	0.04	-0.01	-0.04	0.15
4.	1d	-0.60	0.05	-0.10	-0.09	-0.24	-0.07
5.	1e	-0.47	0.39	-0.20	-0.17	-0.30	0.15

ICM: Ion channel modulator; EI: Enzyme inhibitor; GPCR: G-protein coupled receptor; PI: Protease inhibitor; KI: Kinase inhibitor; NR: Nuclear receptor.

In-silico Toxicity Prediction

The toxicity of the best 5 compounds and Lapatinib were predicted using ProTox II. Hepatotoxicity, carcinogenicity, immunotoxicity, mutagenicity, and cytotoxicity were checked. Only compound 1a is inactive for all 5 toxicities while other 4 compounds were seen to be active for immunotoxicity. However, all 5 compounds were predicted to be better than standard drugs in terms of toxicity. Table-4 displays *in-silico* toxicity results for selected flavonoids.

Table-4: Toxicity Prediction of Standard and Selected Compounds

S. No.	Name	Hepatotoxicity	Carcinogenicity	Immunotoxicity	Mutagenicity	Cytotoxicity
*	Std	A	I	A	I	A
1.	1a	I	I	I	I	I
2.	1b	I	I	A	I	I
3.	1c	I	I	A	I	I
4.	1d	I	I	A	I	I
5.	1e	I	I	A	I	I

A: Active; I: Inactive.

CONCLUSION

As the global burden of cancer is expected to rise significantly by 2040 emphasising the urgent need for continuous research to develop better treatments. The study focused on exploring flavonoids' natural compounds with known health benefits for the potential ErbB4 kinase inhibitors. The experimental approach involved *in-silico* docking studies and ADMET evaluation of a dataset of 329 reported flavonoid compounds. In particular, Inophyllum E stood out as the most promising inhibiting agent with a docking score of -11.4 kcal/mol. The findings of this research impart a foundation for the development of novel ErbB4 kinase inhibitors derived from natural compounds, specifically flavonoids, which could potentially

offer effective and safer alternatives for cancer treatment. Further experimental validation and clinical research is required to ensure the safety and efficacy of these compounds in combating cancer.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to the manuscript, participated in reviewing/editing, and approved the final draft for publication. The research profile of the authors can be verified from their ORCID IDs, given below:

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