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Ligand-Based Drug Design Studies of Different Flavonoids as Potential Inhibitors of BCR-ABL

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Abstract

BCR-ABL is a gene formed by the breakdown and fusion of pieces of two different chromosomes. It causes the production of abnormal blood cells to produce a protein called tyrosine kinase that promotes cancer by allowing uncontrollable cell growth. The BCR-ABL fusion gene is found in most patients with chronic myelogenous leukemia (CML), which is an uncommon type of bone marrow cancer. Several clinical inhibitors have been discovered to lower the BRC-associated CML. But all these clinically practiced inhibitors are potentially harmful to normal cells. Therefore, there is a dire need to identify such novel inhibitors that must be cost-effective, easily available, multitargeted, and less toxic to normal cells. In our study, we have identified some polyphenols, such as Baicalein and Erysubin A, as potential inhibitors of BRC. Dasatinib and Bosutinib are used as controls in our current study. In this context, we investigate thirteen flavonoids as potential ligands through structure-based virtual screening sourced from the PubChem database, with the aim of identifying inhibitors targeting BCR-ABL. Further studied for extra precision, molecular docking was performed using AutoDock Vina, which shows binding affinity of compounds between -6.9 to -10.5 kcal/mol. Erysubin A and Baicalein were the most suitable flavonoids with binding affinities of -10.5 and -10.0 kcal/mol, respectively, compared to clinical inhibitors, i.e., Bosutinib and Dasatinib, having affinities of -7.9 and -8.7 kcal/mol, respectively. Further studies have occupied that Erysubin A and Baicalein have significant interactions with various residues of most sustainable amino acids. The molecular dynamics simulation study indicates the stability and compactness of the protein complex with top drugs, especially Baicalein and Erysubin A. Hence, these results supported the idea that these polyphenols are potential compounds with the ability to inhibit cancer by disrupting the fusion gene activity; hence, further in vitro and in vivo studies are recommended for their development as a novel target to lower the burden of CML.

Keywords: Molecular docking, Flavonoids, BCR-ABL Inhibitor, Drug Discovery, Cancer Treatment

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1. Introduction

CML is a form of myeloproliferative cancer characterized by the massive production of immature WBCs and comprises 15% of all leukemia cases among adults (El-Damasy et al., 2023). A hybrid Philadelphia chromosome (HPC) is formed in patients with leukemia by the transfer of the Abelson (ABL) gene from chromosome 9 to the Breakpoint Cluster Region (BCR) of chromosome number 22. As a result of this transfer, HPC carries a chimeric gene BCR-ABL (Rowley, 1973). The BCR-ABL carries the genetic code for tyrosine kinase (TK) oncoprotein, increasing cell division in CML (Kumar et al., 2016). The C-terminal of the ABL comprises the actin as well as binding sites (Sherbenou et al., 2010). The N-terminal of the ABL comprises two different domains, like SH2 and SH3 (homologous to SRC protein) that maintain the activity of TK (Colicelli, 2010). Any mutation occurring in the gene of the SH2 region may hamper the process of phosphorylation, whereas a mutation in the SH3 region may lead to the process of cell transformation (Blume-Jensen & Hunter, 2001). The N-terminal of the BCR upregulates the reactivity of TK as well as the binding potential of ABL. Serine-TK region of BCR regulates the signaling cascades managed by the ABL (Ren, 2005). A plethora of studies revealed that ABL TK potentially assists in cellular growth and signal transduction (Wang, 1993). BCR-ABL may upregulate the process of cell proliferation and multiple other abnormalities in bone marrow, which consequently affects the production of leukocytes (Baccarani et al., 2006).

Till date, different drugs have been discovered as inhibitors of BCR-ABL. Among them, imatinib is the first-generation FDA-approved inhibitor of BCR-ABL to treat patients with CML (Capdeville et al., 2002). Despite imatinib's initial effectiveness in most CML patients, resistance and drug intolerance hinder its efficacy in about 40% of patients (Pandrala et al., 2022). Imatinib resistance mostly occurs due to mutations within the region of BCR-ABL kinase (Hughes et al., 2006), which affects its appropriate binding with the target (O'Hare et al., 2007). Second-generation inhibitors of BCR-ABL, including dasatinib, nilotinib, and bosutinib, have been used to treat adult patients of CML with resistance to imatinib (El-Damasy et al., 2023). However, second-generation inhibitors failed to overcome the effects of various imatinib-resistant mutations (Vener et al., 2020). In 2012, the 3rd generation inhibitor of BCR-ABL, ponatinib was finally get some success and was approved by the FDA for clinical use. It proved to be an excellent inhibitor of imatinib-resistant mutations; however, some serious concerns arose against the use of ponatinib because it was associated with severe vascular/cardiotoxic effects. Ponatinib is a multi-kinase inhibitor, and it is thought that its cardiotoxic effect is due to its multi-kinase activity and synchronous inhibition of some important kinases that play a key role in cardiovascular function (El-Damasy et al., 2023). Therefore, there is a dire need to design novel inhibitors for BCR-ABL kinase that offer an appropriate approach for the treatment of ALL and CML.

Polyphenolic compounds are heterogeneous groups of plant secondary metabolites. They are further divided into 6 groups (flavonoids, flavanols, isoflavonoids, flavones, flavanones, and anthocyanidins). Interestingly, most flavonoids are derived from natural resources like fruits, vegetables, and different plants, so these compounds can be less toxic, frequently available, cost-effective, and multitargeted (Ashaq et al., 2021). Flavonoids are well known due to their wide range of anticancer activities; they influence the concentration of reactive oxygen species (ROS), arrest the cell cycle at various phases, cause apoptosis and autophagy, and reduce cancer cell proliferation and invasion (Kopustinskiene et al., 2020). By viewing

Table 1: Molecular Formula and Molecular weight of ligands used in this study.

Sr No	Ligands	Nature	Molecular Formula	Molecular Weight (g/mol)
Ct- 1	**Dasatinib-(BMS354825)**	Clinical compounds used as a control	$C_{22}H_{26}ClN_7O_2S$	488.0
Ct-2	**Bosutinib**		$C_{26}H_{29}Cl_2N_5O_3$	530.4
F-1	**Baicalein**	Flavonoids used as potential anti-cancer agent	$C_{15}H_{10}O_5$	270.24
F-2	**Bavachin**		$C_{20}H_{20}O_4$	324.4
F-3	**Erysubin A**		$C_{20}H_{16}O_6$	352.3
F-4	**Isobavachin**		$C_{20}H_{20}O_4$	324.4
F-5	**Nobiletin**		$C_{21}H_{22}O_8$	402.4
F-6	**Tricin**		$C_{17}H_{14}O_7$	330.29
F-7	**Apigenin**		$C_{15}H_{10}O_5$	270.24
F-8	**Acacetin**		$C_{16}H_{12}O_5$	284.26
F-9	**Chyrsin**		$C_{15}H_{10}O_4$	254.24
F-10	**Naringenin**		$C_{15}H_{12}O_5$	272.25
F-11	**Quercetin**		$C_{15}H_{10}O_7$	302.23
F-12	**Galangin**		$C_{15}H_{10}O_5$	270.24
F-13	**Kaempferol**		$C_{15}H_{10}O_6$	286.24

Ct* for Control **F*** Flavonoids

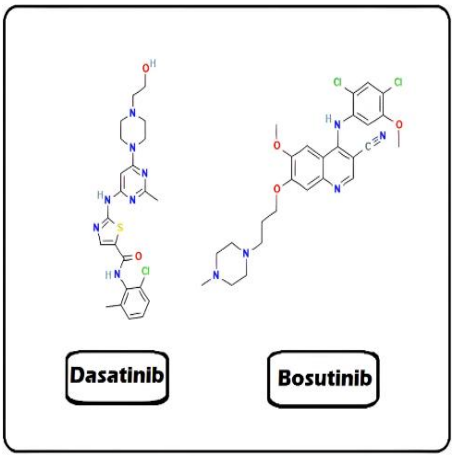

Figure 1 Clinical compounds Dasatinib and Bosutinib used as controls in the current study.

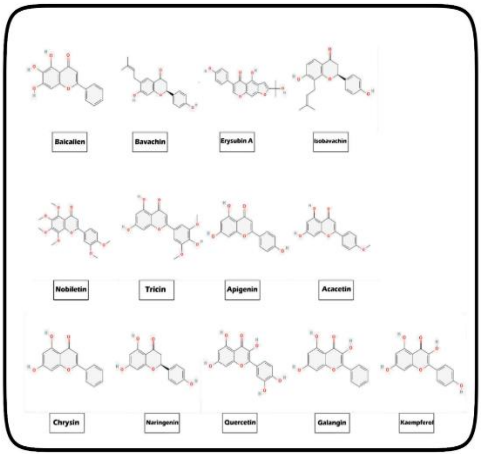

Figure 2 Structural formulas of different flavonoids used as inhibitors of BCR-ABL.

The bioactive potential of flavonoids here in this study, we screened more than 100 flavonoids and identified thirteen of them as potential inhibitors of BCR-ABL by using different bioinformatic tools, as it's the fastest and most cost-effective way to propose any effective molecule as a candidate for a potential novel drug.

2. Materials and Methods

2.1 Ligand Preparation:

Three-dimensional (3D) flavonoids and clinical compounds used as controls were downloaded from the PubChem database (https://www.pubchem.ncbi.nlm.nih.gov/) (Kim et al., 2019). Pymol software was used to make a PDB format of retrieved ligands (DeLano et al., 2002) and then to PDBQT format using AutoDock MGL Tools 1.5.7. (Berman et al., 2000). Figure 1 shows that both clinical compounds, Dasatinib and Bosutinib, are used as controls. 13 best docked flavonoids, Baicalein, Bavachin, Erysubin A, Isobavachin, Nobiletin, Tricin, Apigenin,

Acacetin, Chrysin, Naringenin, Quercetin, and Galangin are shown in Figure 2. The molecular weight and molecular formula of all the ligands is given in Table 1.

2.2 Protein Preparation

Crystal structure (3D) of BCR-ABL oncoprotein oligomerization domain (PDB Id: 1K1F) (Figure 3) was downloaded from Protein Data Bank (https://www.rcsb.org/) (Berman et al., 2000). For further preparation, the protein structure was opened in AutoDock MGL Tools 1.5.7 (Morris et al., 2009). Using AutoDock MGL Tools 1.5.7, molecules of water were deleted, polar hydrogens and Kollman charges were added. After Preparation, the receptor protein was saved in the PDBQT format. The locality of active sites for ligand docking was determined by generating the 3D map of the grid box on the receptor region of the macromolecule using AutoDock MGL Tools 1.5.7 (Morris et al., 2009). The grid size was determined at 40×40×40 xyz points with 0.375 Å spacing, and the grid center was designed as x = 37.000, y = 46.026, z = -14.901.

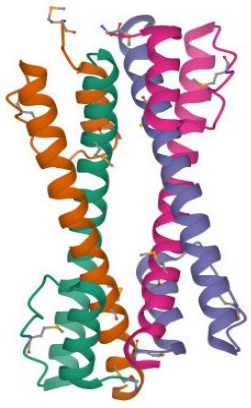

Figure 3 Crystal structure of the BCR-ABL oncoprotein oligomerization domain.

2.3 Molecular Docking and Visualization

AutoDock vina (Trott et al., 2010) program was utilized for molecular docking using the PDBQT form of BCR-ABL and selected ligands. Visualization of the BCR-ABL oncoprotein oligomerization domain with interacting ligands was performed using BIOVIA Discovery Studio 2021 (Biovia et al., 2016).

2.4 Drug Likelihood and Toxicity Prediction

The potential inhibitors or best docked ligands are determined by the results, expressing their binding energies. However, ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) properties provide significant methodologies being processed in drug discovery and development (Jia et al., 2022). The Lipinski rule of five determined efficiency, metabolism, and safety by calculating different parameters. Toxicology predictions highlight the quantity of small molecules that could be tolerated by human and animal models (Pires et al., 2015). ADMET properties, Lipinski rule of five, and toxicity prediction for decided ligands were calculated as reported by Yousaf et al. (2024).

Table 2: Results of molecular docking showing binding energies and different interactions of ligands with BCR-ABL.

Ligands	Binding Energies (**kcal/mol**)	Hydrogen Bonding	Hydrophobic Interactions	Electrostatic Interactions
Dasatinib-(BMS354825)	-8.7	ARG43, GLN47 ASN50, GLN14 ASN50, GLN47	ARG43, ILE42 LYS39, ALA40	ARG43
Bosutinib	-7.9	ARG43, GLN14 ASN50	ARG43, LYS39 ILE42	ARG43
Baicalein	-10.0	ARG43, ASN50 ASN50, GLN14 GLU46	ARG43, LYS39	ARG43
Bavachin	-8.7	ARG43, LYS39 ALA13	ARG43, LYS39 ILE42	ARG43, GLU36
Erysubin A	-10.5	ARG43, ASN50 GLU46, LYS39	LYS39	GLU36, GLU36
Isobavachin	-7.7	ARG43, GLN14	ARG43	GLU46
Nobiletin	-6.9	ARG43, GLN14 GLU46, GLN47	ARG43	GLU46
Tricin	-8.0	GLN47, ASN50 ALA13, LYS39 ASN50, ARG43	LYS39, ARG43	
Apigenin	-7.7	ARG43, LYS39 GLU46, GLU36 ALA13	ALA40, LYS39 ARG43	ARG43, GLU36
Chrysin	-7.7	GLU36, LYS39	LYS39, ARG43	ARG43, GLU36
Naringenin	-8.1	LYS39, ARG43 GLN14, GLU46	ARG43, LYS39	ARG43
Galangin	-7.6	ARG43, ASN50	ARG43, LYS39	GLU46
Quercetin	-8.0	ARG43, GLN14	ARG43, LYS39	ARG43
Acacetin	-8.0	ARG43, ASN50 GLN14, GLU46 GLU36	LYS39, ARG43	ARG43
Kaempferol	-7.9	ARG43, LYS39	LYS39, ARG43 ALA40, ILE42	ARG43, GLU36

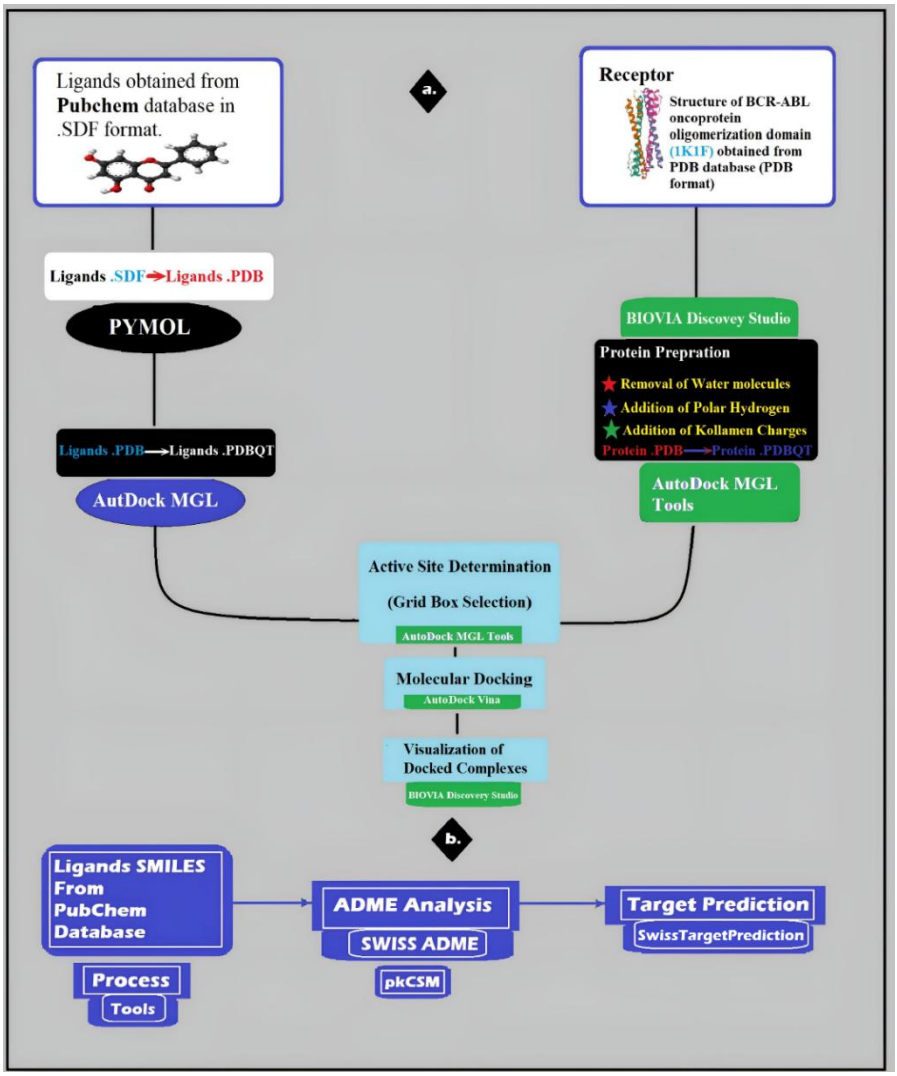

Figure 4 The overall flowchart of the current study.

2.5 Target Prediction

Target prediction is a significant tool to assess the phenotypical side effects or potential cross-reactivity due to small biomolecule action (Gfeller et al., 2014; Keiser et al., 2007). Swiss Target Prediction (https://www.swisstargetprediction.ch) (Vener et al., 2020) was used to predict the possible targets with *Homo sapiens* by using PubChem SMILES of ligands as input. The overall flowchart for the current study is shown in Figure 4.

2.6 Molecular dynamics simulation

The stability of the protein-ligand complex was further checked by using the analysis of molecular dynamics (MD) simulations. These simulations were conducted over a 50 ns period using the GROMOS96 43a1 force field within the GROMACS 2019.2 software package, facilitated by WebGRO for molecular

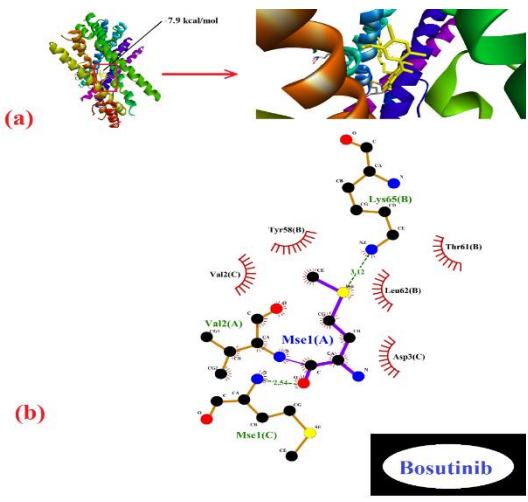

Figure 5 Bosutinib with its binding sites, bindin energies, and interactions with amino acid residu through docking with BCR-ABL.

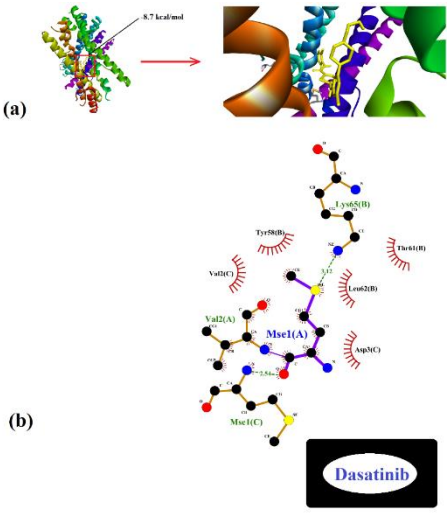

Figure 6: Dasatinib with their binding sites, binding energies and interactions with amino acid residues through docking with BCR-ABL.

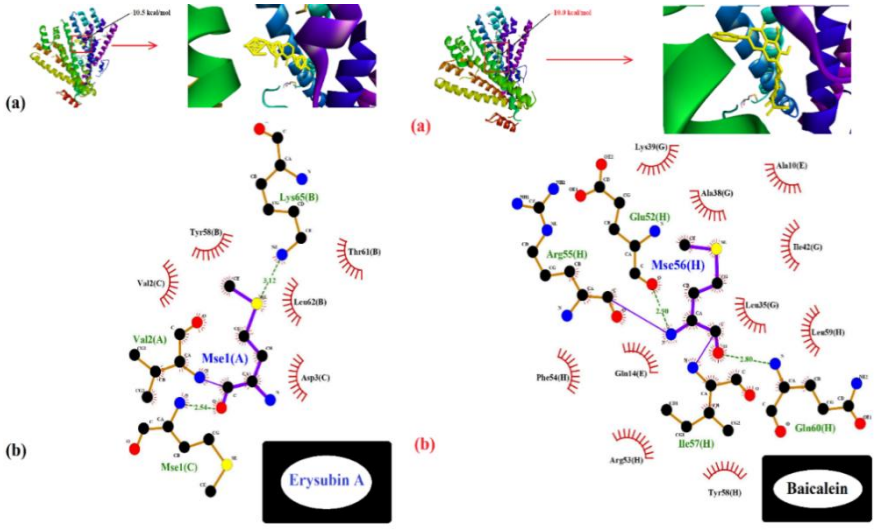

Figure 7: Erysubin A and Baicalein with their binding sites, binding energies and interactions with amino acid residues through docking with BCR-ABL.

simulations, as described by Yousaf et al. (2024).

3. Results

The binding energies and interactions of all ligands with BCR-ABL are given in Table 2. In the current study, we found Erysubin A and Baicalin as potent lead inhibitors of BCR-ABL, as they show better binding affinities as compared to control drugs.

For the ligand displacement at the binding site of protein, the hydrogen bonds are the most important, and their presence in the docked complex indicates the good interaction between selected ligands and

Table 3: Shows different parameters of Lipinski's rule for selected flavonoids against BCR-ABL.

LIGANDS	HBR (Hydrogen Bond acceptor)	HBD (Hydrogen Bond donor)	Log P	Lipinski Rule	
				Drug likeliness	Violation
Dasatinib-(BMS354825)	6	3	2.80	Yes	Zero
Bosutinib	7	1	4.28	Yes	One; MW>500
Baicalein	5	3	2.24	Yes	Zero
Bavachin	4	2	3.53	Yes	Zero
Erysubin A	6	3	2.85	Yes	Zero
Isobavachin	4	2	3.53	Yes	Zero
Nobiletin	8	0	3.02	Yes	Zero
Tricin	7	3	2.15	Yes	Zero
Apigenin	5	3	2.11	Yes	Zero
Acacetin	5	2	2.52	Yes	Zero
Chrysin	4	2	2.55	Yes	Zero
Naringenin	5	3	1.84	Yes	Zero
Quercetin	7	5	1.23	Yes	Zero
Galangin	5	3	1.99	Yes	Zero
Kaempferol	6	4	1.58	Yes	Zero

protein, leading to the inhibition of the targeted protein (Aanouz et al., 2020; Aarjane et al., 2020). All studied flavonoids contain H-bonding with amino acids, a maximum of six hydrogen bonds present in multiple polyphenols, while a minimum of hydrogen bonding is present in chrysin, which has only two H-bonds with amino acids. Table 2 has shown that, the binding affinity of Bosutinib was -7.9 kcal/mol and it formed three hydrogen bonds with amino acids residues by ARG43, GLN14, ASN50 and three hydrophobic and one electrostatic interaction, while Dasatinib has binding affinity -8.7 kcal/mol and it formed six hydrogen bonds with amino acid residues by ARG43, GLN47, ASN50, GLN14, ASN50, GLN47 and three hydrophobic and one electrostatic interaction. Figure 5 and 6 depicted the binding energies and amino acid residues in 3D and 2D diagram of docked ligands act as a control i.e., Dasatinib and Bosutinib.

All studied compounds demonstrated strong interactions with the binding site residues of BCR-ABL; their binding affinity ranges between -6.9 kcal/mol to -10.5 kcal/mol. Erysubin A shows the

highest affinity as -10.5 kcal/mol, while Baicalein shows next, his a binding affinity was -10.0 kcal/mol. Erysubin A formed four hydrogen bonds, one hydrophobic and three electrostatic interactions with amino acid. While Baicalein has formed five hydrogen bonds, two hydrophobic and one electrostatic interaction with the amino acid, as shown

Table 4: Toxicity properties for controls and selected flavonoids used as BCR-ABL inhibitors.

Ligand	ADMET Toxicity	Max. tolerated dose (human)(log mg/kg/day)	hERG I inhibitor	hERG II inhibitor	Oral Rat Acute Toxicity(mol/kg)	Oral Rat Chronic Toxicity (log mg/kg_bw/day)	Liver Toxicity	Skin Sensitisation	*T. pyriformis* toxicity (log ug/L)	Minnow toxicity (log mM)
Dasatinib	No	No	No	No	No	No	No	No	No	No
Bosutinib	No	0.066	No	Yes	2.736	0.421	Yes	No	0.323	1.3
Baicalein	No	0.498	No	No	2.325	2.645	No	No	0.42	1.25
Bavachin	No	-0.445	No	No	2.27	1.227	No	No	0.909	0.76
Erysubin A	Yes	0.596	No	Yes	2.507	1.444	No	No	0.296	1.195
Isobavachin	No	-0.157	No	No	2.394	1.435	No	No	0.853	1.389
Nobiletin	No	0.443	No	No	2.459	0.82	No	No	0.315	0.686
Tricin	No	0.351	No	No	2.229	1.82	No	No	0.329	1.754
Apigenin	No	0.328	No	No	2.45	2.298	No	No	0.38	2.432
Chrysin	No	0.016	No	No	2.289	0.955	No	No	0.535	1.746
Naringenin	No	-0.176	No	No	1.791	1.944	No	No	0.369	2.136
Galangin	No	0.333	No	No	2.45	2.323	No	No	0.382	2.385
Quercetin	No	0.499	No	No	2.471	2.612	No	No	0.288	3.721
Acacetin	No	0.09	No	No	2.22	1.259	No	No	0.422	1.00

in Figure 7.

3.1 Drug-likeliness

The incompatible interaction between inhibitors and protein or enzyme may not guarantee the inhibitor stability as a drug; therefore, ADME analysis plays a critical role during the drug formation process. ADME analysis depends on Lipinski's rule of five, which is a thumb rule for the evaluation of drug likeness (Lipinski, 2004). This rule provides a vital parameter for the evaluation of pharmacokinetic parameters of drugs such as absorption, distribution, metabolism, and excretion

(ADME) for designing and developing of drug (Ertl et al., 2000). According to the rule, any molecule can be studied for drug development if it has a Molecular weight ≤ 500 Da, a Number of H-Bond donors (HBD) ≤ 5, a Number of H-Bond acceptors (HBR) ≤ 10, LogP (lipophilicity) ≤ 5 (Veber et al., 2002). Compounds having fewer than three of these conditions are unlikely to be assigned as an orally bioavailable drug and will cause trouble on ingestion (Mendis et al., 2011).

The molecular characters of all ligands were weighed by SwissADME (Talele et al., 2010). The results of physicochemical

properties in Table 3 revealed that the molecular weight of selected flavonoids ranged from 254.24 Da (chrysin) to 402.4

Da (Nobiletin) (≤ 500), number of HBD from 2 (Bavachin, Isobavachin, Chrysin and Acacetin) to 5 (Quercetin) (≤ 5), number of HBR from 4 (Bavachin, Chrysin and Isobavachin) to 7 (Tricin and Quercetin) (≤ 10) and the value of LogP varied from 1.23 (quercetin) to 3.53 (Bavachin and Isobavachin) (≤ 5), results differs for the controls i.e., Dasatinib and Bosutinib. Dasatinib has a molecular weight of 488 (g/mol) (≤ 500), number of HBD 3 (≤ 5), number of HBR 6 (≤ 10), and value of LogP 2.80 (≤ 5), and obeys all Lipinski rules with zero violation. Our other control, i.e., Bosutinib, has the molecular weight of 530 (g/mol) (> 500), number of HBD 1 (≤ 5), number of HBR 7 (≤ 10), and value of LogP 4.28 (≤ 5), and obeys Lipinski rule with one violation, i.e., (M.W > 500). The overall data showed that all selected flavonoids were observed to obey Lipinski's rule. Table 3 shows the values of HBR, HBD, Log P, and Lipinski violation, while the molecular weight of each compound is mentioned in Table 1.

3.2 Toxicity Prediction

Summary of pkCSM predictions (Pires et al., 2015) has been displayed in Table 4. The results suggested that all investigated compounds do not possess any ADMET toxicity. Positive prediction of ADMET represents the carcinogenicity of a compound (Pires et al., 2015; Xu et al., 2012). The highest tolerated dose (HTD) for humans is estimated to be the toxic range of any drug, which is mentioned as a starting dose during phase 1 clinical trials (Pires et al., 2015). Values of HTD for all compounds were lower except Quercetin and Erysubin A (0.499 and 0.596 mg/kg/day, respectively), which have slightly higher values than the threshold level of 0.477 log mg/kg/day. Inhibition of human ether-a-go-go gene (hERG) was not observed by all of the compounds, but for hERG II, Inhibition was predicted for only Erysubin A. hERG lowers the activity of potassium channels, which leads to the development of long QT syndrome (Vandenberg et al., 2001; Chiesa et al., 1997). hERG potassium channels permanently banned the use of several drugs (Pires et al., 2015). Oral rat chronic toxicity was evaluated from 0.955 log mg/kg_bw/day (chrysin) to the highest value of 2.645 log mg/kg_bw/day for Baicalein. None of the compounds was found to be positive for skin and liver toxicity. In this study, no compound showed skin sensitization. For toxicity against *T. pyriformis* (a protozoan), a compound having a predicted value of pIGC50 (negative algorithm of concentration which is needed to inhibit 50% of growth in log μg/L) < −0.5 log μg/L is regarded as toxic. No compound showed the value in the described range of T. pyriformis toxicity. For Fathead Minnows fish, the lethal concentration (LC50) causing 50% mortality in the Minnows test group, LC50 < 0.5 mM (log LC50 < −0.3) is considered to show acute toxicity (Pires et al., 2015; Cheng et al., 2011). No compound was found to exhibit Minnow toxicity as a result of toxicity analysis.

3.3 Target Prediction

The target prediction analysis for the compounds used as controls in our current study, i.e., Dasatinib and Bosutinib, and

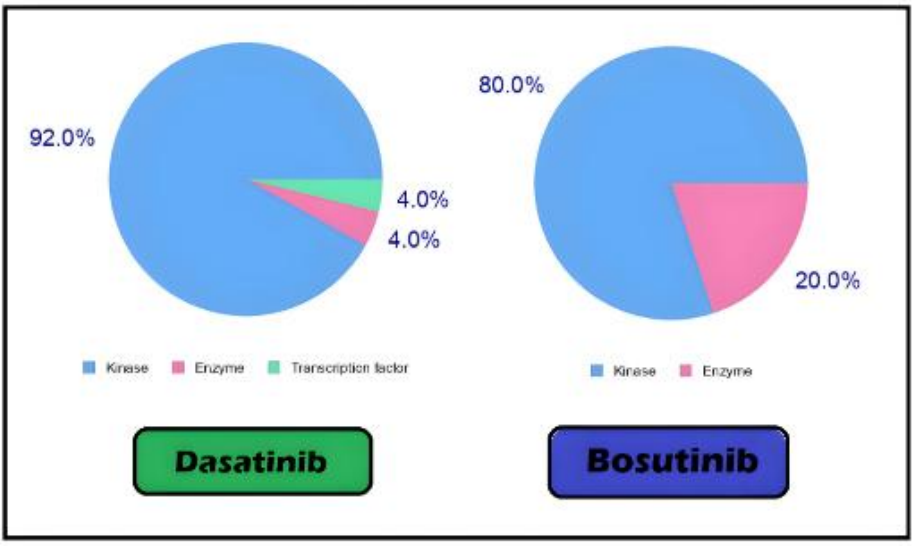

Figure 8: Top twenty-five (25) targets predicted by the SwissTargetPrediction database for Dasatinib and Bosutinib used as a control in our current study

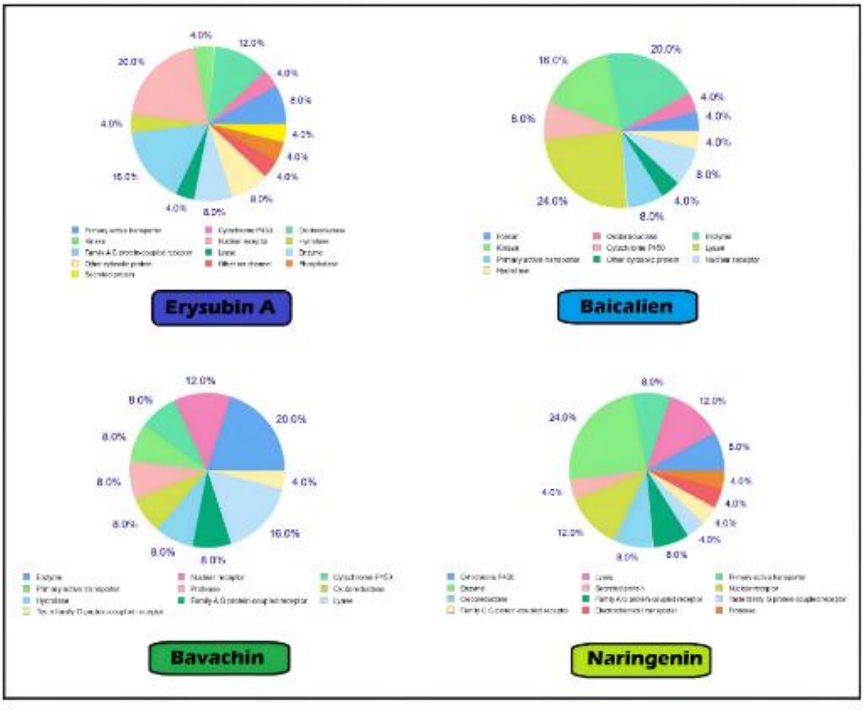

Figure 9: Top twenty-five (25) targets predicted by the SwissTargetPrediction database for top top-ranked flavonoids used in our current study

our selected flavonoids used as ligands to inhibit BCR-ABL activity, was performed by Swiss Target Prediction software, and the top twenty-five observations were

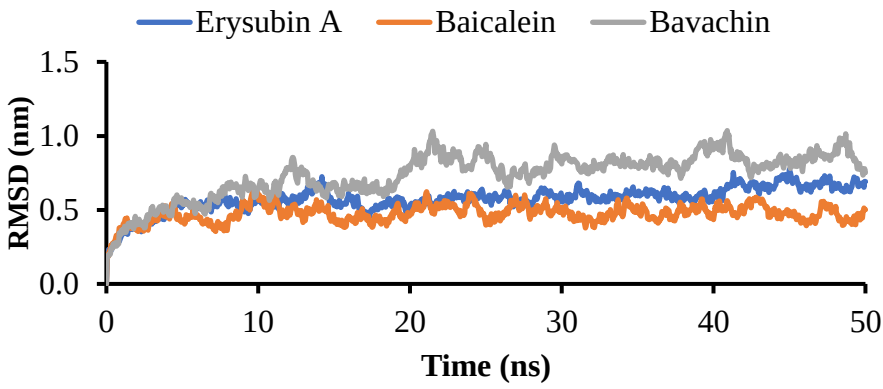

Figure 10: RMSD values for Erysubin A, Baicalein, and Bavachin in complex with BCR-ABL over a 50 ns molecular dynamics (MD) simulation. The RMSD values indicate the structural stability of each compound-protein complex.

displayed as pie-charts in Figure 8. For Dasatinib, the pie-chart predicted 4% enzyme, 92.0% of kinase, and 4.0% of Transcription factor as target. Analysis predicted that Bosutinib targeted the 80% kinase and 20% enzyme. The figure shows the pie charts of top twenty-five observations of compounds used as a control.

For Erysubin A, SwissTargetPrediction software predicted the top twenty-five observations, that are displayed as pie-chart, predicted 20% of nuclear receptor, 16% family A-G protein-coupled receptor, 12% of oxidoreductase, 8% of primary active transporter, 8% of enzyme, 8% of other cytosolic protein, 4% of each lyase, other secreted protein, phosphatase, cytochrome P450, other ion channel, nuclear receptor and hydrolase as a target as shown in figure 9. The pie chart of Baicalein for the top twenty-five observations predicted 4% eraser, 16% kinase, 8% primary active transporter, 4% hydrolase, 4% oxidoreductase, 8% cytochrome P450, 4% other cytosolic protein, 20% enzyme, 8% nuclear receptor, and 24% lyase as a target. Bavachin for the top twenty-five observations in the pie-chart targeted 20% enzyme, 16% lyase, 8% hydrolase, 8% primary active transporter, 4% Taste family G protein-coupled receptor, 12% protein receptor, 8% protease, 8% Family A G protein-coupled receptor, 8% cytochrome P450, and 8% oxidoreductase as by pie-chart. From top four, our last ligand i.e., Naringenin got the target prediction from Swiss Target Prediction software for the top twenty-five observations that displayed 24% of enzyme, 8% of cytochrome P450, 12% of nuclear receptor, 12% of lyase, 8% oxidoreductase, 4% family C G protein-coupled receptor, 4% secreted protein, 8% A G protein-coupled receptor, 4% electrochemical Transporter, 4% protease and % taste family G protein-coupled receptor.

The average probability scores for our selected flavonoids were around zero. Previous studies have shown that a probability value greater than zero was considered to depict a reasonable drug-ligand interaction (Gfeller et al., 2014; Thomas et al., 2020). The probability score of the analyzed ligands indicated that they have better attraction towards the target of the specific binding site.

3.4 MD simulation

Root mean square deviation (RMSD) values indicate the stability of each

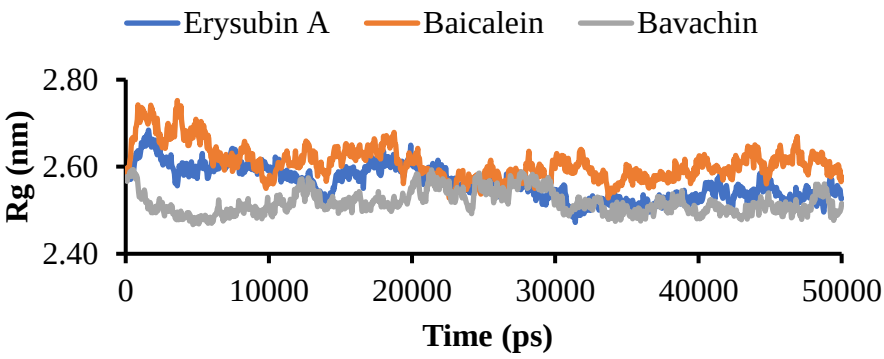

Figure 11: Rg values for Erysubin A, Baicalein, and Bavachin in complex with BCR-ABL over a 50 ns MD simulation. The Rg values reflect the compactness of each complex.

compound within the protein complex. Erysubin A has a mean RMSD of 0.573 nm, ranging from 0. 0000005 nm to 0.772 nm. Baicalein exhibits a lower mean RMSD of 0.474 nm, with a maximum of 0.640 nm. In contrast, Bavachin shows the highest mean RMSD at 0.736 nm with a range of 0.0000004-1.0357153 (Figure 10).

The radius of gyration (Rg) assesses the compactness of each compound-protein complex. Erysubin A maintains a mean Rg of 2.562 nm, with values ranging from 2.472 nm to 2.684 nm. Baicalein shows slightly higher compactness, with a mean Rg of 2.609 nm. Bavachin has a mean Rg of 2.518 nm, which may indicate a somewhat less compact interaction (Figure 11).

4. Discussion

ABL and BCR genes are found on chromosomes number 9 and 22, respectively. In BCR-ABL induced leukemia, the translocation of BCR and ABL genes occurs between chromosomes 22 and 9, thus resulting in the generation of the BCR-ABL oncogene, which is the leading cause of CML (Amarante-Mendes et al., 2022). Multiple therapeutic agents are available to minimize the burden of CML, but their safety and reliability is a major challenge. The idea of using natural products for the treatment of cancer has been remarkably proven (Yousaf et al., 2024). This study will mainly focus on identifying new inhibitors of BCR-ABL from a wider variety of flavonoids by using different computational methods. In this study, different flavonoids have been used as anti-cancer agents against the activity of TK, which leads to the production of uncontrolled cells and leads to bone marrow cancer mostly. 90% of CML patients have the BCR-ABL oncogene in them. 13 flavonoids: Baicalein, Bavachin, Erysubin A, Isobavachin, Nobiletin, Tricin, Apigenin, Acacetin, Chrysin, Naringenin, Quercetin, Galangin, Kaempferol were used as potential anti-cancer agents to inhibit tyrosine kinase activity. Molecular docking was performed to calculate the binding affinity of flavonoids used as a ligand in this study. Clinical compounds, i.e., Dasatinib and Bosutinib, score binding affinity -8.7 and -7.9 kcal/mol, respectively. Results of molecular docking ranked flavonoids based on binding affinity as: Erysubin A (-10.5 kcal/mol > Baicalein (-10.0 kcal/mol) > Bavachin (-8.7 kcal/mo) > Naringenin (-8.1 kcal/mol) > Quercetin, Acacetin, Tricin (-8.0 kcal/mol), Kaempferol (-7.9 kcal/mol) >

Isobavachin, Apigenin, Chrysin (-7.7 kcal/mol) > Galangin (-7.6 kcal/mol) > Nobiletin (-6.9 kcal/mol). Erysubin A and Baicalein were front-line compounds with the top binding affinity among all. Therefore, supported as a potential anti-cancer agent. Moreover, results of drug likeness and toxicity analysis further supported the idea to propose flavonoids as a potent inhibitor of BCR-ABL.

Finally, the MD simulation data on RMSD and Rg values for Erysubin A, Baicalein, and Bavachin in complex with BCR-ABL provide insights into the stability and structural behavior of these compounds. The RMSD values reveal stability within the BCR-ABL binding site, with lower values indicating a stable interaction and higher values suggesting increased fluctuation. Following equilibration after the initial rise, the complexes displayed consistent stability with only minor fluctuations, which serve as strong indicators of system stability (Kuzmanic & Zagrovic, 2010). Baicalein shows the lowest average RMSD, implying a relatively stable binding configuration, while Erysubin A, with a slightly higher RMSD, also demonstrates stable interaction. In contrast, Bavachin's higher RMSD suggests a more flexible or less stable binding interaction, as evidenced by its greater structural deviations. The Rg values reflect the compactness of the complex structures, where a lower Rg generally suggests a more compact and potentially tighter binding interaction. Baicalein has the highest Rg, followed closely by Erysubin A, indicating both form relatively compact complexes with BCR-ABL. Bavachin exhibits slightly lower compactness, consistent with a more flexible interaction with the protein. In summary, these RMSD and Rg data suggest that Baicalein and Erysubin A establish relatively stable and compact interactions with BCR-ABL, while Bavachin shows more flexibility and reduced stability. These differences in stability and compactness may be significant for understanding the varying binding efficiencies or potential effectiveness of these compounds when targeting BCR-ABL.

5. Conclusion

The ABL gene from chromosome 9 joins to the BCR gene on chromosome 22, to form the BCR-ABL. It causes the production of abnormal cells that upregulate the TK activity, which consequently causes uncontrolled cell growth. 90% of patients having CML have the BCR-ABL oncogene. Several antioncogenic therapeutics have been approved for the treatment of CML, but their success is limited due to their toxic nature. Flavonoids have been well known due to their lot of bio-medicinal properties. 13 flavonoids: Baicalein, Bavachin, Erysubin A, Isobavachin, Nobiletin, Tricin, Apigenin, Acacetin, Chrysin, Naringenin, Quercetin, Galangin, Kaempferol were used as potential anti-cancer agents to inhibit tyrosine kinase activity. Erysubin A and Baicalein were front-line polyphenols with the top binding affinity among others. Therefore, both were supported as potential anti-cancer agents and pointed towards further in vitro and in vivo studies to confirm their mechanism of action and ensure their development as a novel anticancer agent. We recommend the several in vitro analyses, like biochemical kinase inhibition assays (to measure IC_{50}), cellular phosphorylation assays such as pCRKL or pSTAT5 (to confirm target engagement), and cell-viability assays in BCR-ABL positive cell lines like K562 to validate our in silico BCR-ABL inhibition results, several standard in vitro assays can be used, Additional apoptosis or colony-formation assays may further support the inhibitory activity.

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