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INSILICO MOLECULAR DOCKING AND ADME/T STUDIES OF FLAVONOL COMPOUNDS AGAINST SELECTED PROTEINS INVOLVED IN INFLAMMATION MECHANISM

Narendra Pentu[1]*, Ajitha Azhakesan[2], Pasupuleti Kishore Kumar[3]

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ABSTRACT

Background: Using computational tools in drug discovery advanced the research in identifying new drug candidates for the benefit of the pharmaceutical industry and assessing the safety and pharmacokinetic profiles of phytochemicals. Understanding the inflammatory mechanism is not possible, but inflammatory signal transduction by cytokines can be mitigated by using the flavonoid class of drugs like flavonols. **Methodology:** A molecular docking study of flavonol compounds with proteins linked with inflammation was carried out using the AutodockVina program. SwissADME and pkCSM modules were used to assess the pharmacokinetic features of plant products. Compared to commercially available NSAIDs, flavonols had more excellent molecular docking scores. **Results:** Calculation of ADME features of flavonols with no carcinogenicity and low oral acute toxicity level. Compared to anti-inflammatory medicines, the Rutin docking score against COX-I (-8.7 kcal/mol) and the Galangin docking score against COX-II enzymes (-9.4 kcal/mol) had higher values. **Discussion:** Molecular docking studies exhibited the highest docking score for COX-I is Rutin -8.7 Kcal/mol and hydrogen bond with THR-89, PRO-84, LS-468, GLY-471, PHE-470. The highest docking for COX-II is Galangin -9.4 Kcal/mol and hydrogen bonding with VAL-349 and TYR-385. ADME/T studies were performed for all the flavonols. Rutin has the highest violations in drug-likeliness studies. **Conclusion:** Flavonols may be more effective anti-inflammatory medicines than commercial medications. By modifying the pharmacokinetic features of plant products through diverse formulation strategies, we can get these phytochemicals to their target sites with fewer adverse effects.

INTRODUCTION

Inflammation is defined as a tissue response to the injury (Celsus in the 1st century AD), and the symptoms caused include redness, swelling, pain and heat due to hyperemia, infiltration of proteins, nerve-ending activation, and changes in the chemical reactions. Further, it leads to infection. The inflammatory

[1]*Department of Pharmaceutics, CMR College of Pharmacy, Hyderabad, India*
[2]*Department of Pharmaceutical Chemistry, Sri Ramachandra Faculty of Pharmacy, Sri Ramachandra Institute of Higher Education and Research (Deemed to be University), Chennai, India.*
[3]*Department of Pharmacology, CMR College of Pharmacy, Hyderabad, India*

For Correspondence:** **narendrapentu@gmail.comn

cascade involves numerous activations and the manufacture of inflammatory agents such as amines, bioactive lipids and peptides, glycoproteins, endotoxins, and response compounds from diverse human body parts. These signalling mechanisms in the inflammatory process activate leukocytes, which then travel to the wounded region via chemotaxis. Leukocytes generate cytokines that drive inflammatory reactions. Scholars are interested in understanding how dysregulated inflammation occurs in different autoimmune diseases. Most of these parameters are regulated by inhibiting endothelial membrane factors, pro-inflammatory chemicals, and platelet aggregation mechanisms. Anti-inflammatory medication is explicitly implicated in any mechanisms that impede the above mentioned components. Apart from the medication's inhibiting effects, our bodies have defence mechanisms like eicosanoids, which act as mediators of inflammation and intensify histamine, bradykinin, and blood proteins that phagocyte the inflammatory agent to eliminate antigens and halt the inflammatory process. Inflammation is the underlying cause of many chronic diseases and their symptoms; enzymes evolved in the inflammation process are inhibited by several mechanisms. Treatment includes relapsing the symptoms but not eradicating the underlying cause of the disease. Treatment requires several phases, including NSAIDs, synthetic glucocorticoids, and biologics. Several adverse effects have been observed, including gastrointestinal, skin, liver, renal, and medication intolerance[1,2].

Inflammation occurred at the cell molecular level. This inflammatory action was signalled and further propelled by the inflammatory cytokines. There are several pathways in the mechanism of inflammation, but proteins like Cyclooxygenase-I & Cyclooxygenase-II compounds cause most of the inflammatory reactions. Abnormal expression of COX-II is important in inflammation. Selective inhibition of COX-II by COX-II inhibitors prevents inflammation, proliferation and angiogenesis. COX-II inhibitors have been shown to act synergistically with chemotherapeutic and targeting agents[3]. Numerous studies showed the suppression of COX-II by quercetin flavonol. Most NSAIDs act against the COX enzymes and show adverse effects like GIT irritation and renal toxicity. The non-specific side effect exerted by the COX-2 inhibitors is due to the inhibition of the Physiologically important COX-I enzyme. The availability of Over-the-counter drugs, specifically in NSAID's division, increased. It is important to consider the safety and efficacy of the drugs and how best we can reduce the side effects. 14-25% of users of NSAIDs are encountered gastric and duodenal ulcers[4]. NSAID usage after the age of 70 years creates a serious health risk in GIT. These drugs interfere with the Prost gland's production in the inflammatory pathways, which leads to reduced mucosal protection by reducing the effectiveness of the mucus-bicarbonate barrier, gastric acid and pepsin. The expression of COX-I enzymes in various tissues like the stomach, platelets and kidneys. COX-2 expression is undetectable in most of the tissues. Most NSAIDs are non-selective, in which the action against the COX-II adversely affects the COX-I, which is very important in most biological tissues. Unrelated and non-specific side effects exerted by classical NSAIDs are due to the inhibition of the physiologically important cyclooxygenase-1 (COX-1) enzyme.

Several studies suggested that flavonols involve the metabolism of arachidonic acid to interrupt the formation of inflammatory proteins [5]. COX-I and COX-II Proteins are the major contributors to the production of the inflammation cascade. Blocking these proteins may hinder the inflammatory mechanism.[6,7]. Flavonols are a class of compounds that belong to the polyphenols. These compounds include Quercetin, Kaempferol, Myricetin, Galangin and Rutin. Phenolic compounds present in plants for pigmentation, growth, reproduction, resistance to pathogens[8]. Most of the dietary substances like fruits and vegetables consist of polyphenols[9]. Polyphenols are the potential candidates to act against Oxidation[10,11], Inflammation[12] and proliferation of cancer cells[13–15]. Flavonol compounds are beneficial for the treatment of anti-inflammatory condition[16]. COX-II enzyme plays a crucial role in multiple pathological conditions, including inflammation, tissue injury, angiogenesis and tumorigenesis. The structural arrangement of the flavonols plays a key role in their orientation to attach with the proteins, mainly double bond at C2-C3, carbonyl group addition at C4, and Hydroxyl groups at C5 and C7. SAR of flavonols confirms the binding and blocking capacity against the inflammatory proteins, but there is no evidence to show the exact match of the activity.

Molecular docking is a computational procedure that efficiently predicts the noncovalent binding of a macromolecule and ligand. The study aims to predict the bound confirmations and the binding affinity[17,18]. The present work aims at the molecular docking studies of Flavonols and against the proteins involved

in the inflammatory reactions. Their low oral bioavailability limits the clinical use of the flavonols. The ADME studies depict the Physical properties and possibility of the drug's availability at the site of action. Lipinski's rule of 5 is a computational tool to describe the nature of the drug to reach the site of action[19,20].

Flavonols consisting the basic structure of diphenyl Propane (C6-C3-C6). Depending on the structure difference on the C ring, flavonoids are mainly divided into eight subclasses, including flavones, flavonols, flavanones, flavanonols, isoflavones, anthocyanins, chalcones and flavan-3-ols [21,22].

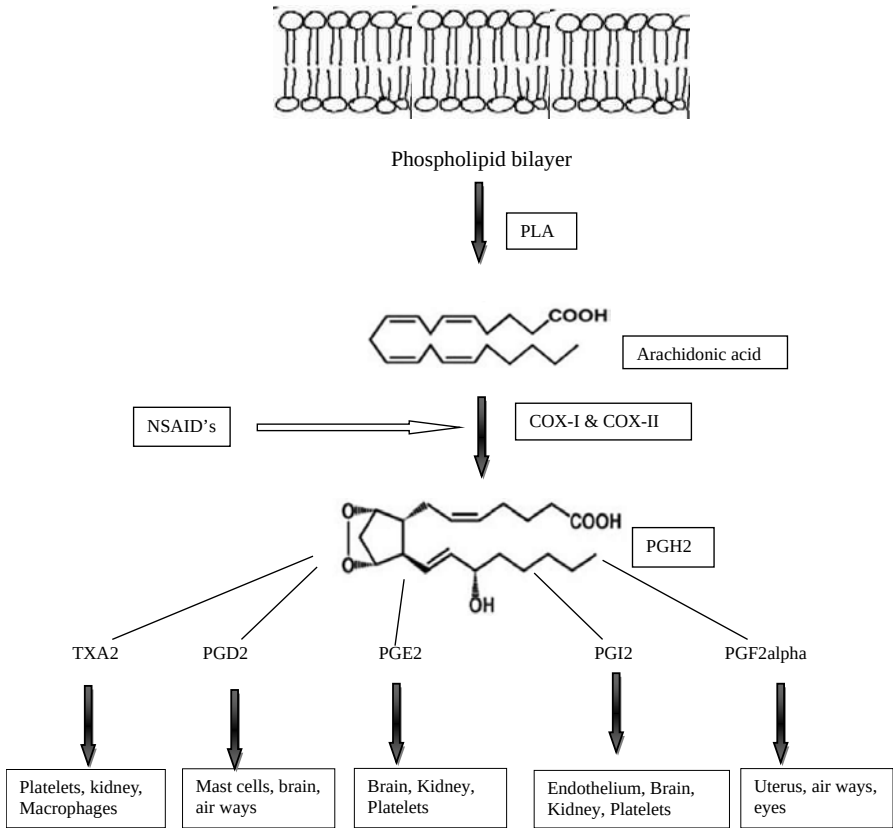


Figure 1: Molecular mechanism of COX-I & COX-II

MATERIALS AND METHODS

The ligands of Flavonols 3D structure were downloaded from PubChem, https://pubchem.ncbi.nlm.nih.gov. In SDF form, receptor molecules are downloaded from the PDB site (https://www.rcsb.org/). Tools like Biovia discovery software https://discover.3ds.com/discovery-studio-visualizer-download, Autodock vina (the software is available from m http://vina.scripps.eduand), SwissADME and pkCSM are used to predict the pharmacokinetic properties of the molecules are freely available software available in internet source. ChemDraw Professional 16.0 software is used to draw the structures of the flavonol compounds.

SwissADME

SwissADME is a freely available software for computing the drug molecules' physicochemical descriptors and ADME parameters. SwissADME was developed and maintained by the Molecular modelling group of the SWISS Institute of Bioinformatics. Initially, we need to draw the structure/ write the SMILES (Simplified molecular input line entry systems) string of the drug molecule in the given square boxes using the following link: http://www.swissadme.ch/. System specifications are Windows 10, 64-bit with an Intel ® Core TM i5-6200U CPU @2.30 GHz[29–30].

Physicochemical descriptors of the drug molecules include the partition coefficient, molecular weight, and total polar surface area. This software predicts ADME/T properties like absorption, Distribution, Metabolism, Excretion, and Toxicity. Drug-related pharmacokinetic parameters data are used to check the drug's likeliness. Lipinski's Rule of Five is used to check properties like molecular weight, partition coefficient, hydrogen bond donors, and hydrogen bond acceptors to estimate the oral bioavailability of the drug candidates [31].

pkCSM

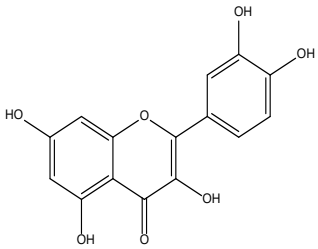
Predicting small molecules' pharmacokinetic properties using graph-based signatures is performed in the pkCSM software. ADME properties are key in estimating bioavailability and drug release kinetics, which are helpful in drug design. A freely available web server (http://structure.bioc.cam.ac.uk/pkcsm) provides an integrated platform to know the kinetic and toxic properties of the drug molecules [32-34].

AutodockVina

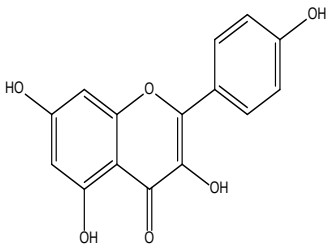
AutodockVina is an open-source software designed and implemented by Dr. Oleg Trott in the CCSB at the Scripps Research Institute. Vina runs with Java and Python programming with both Linux and Windows software with all 64-bit systems. AutodockVina is one of the docking engines of the Autodock suite. Vina's input and output files are in PDBQT format, which is generated and visualized by the MGL tools. Vina is used for Blind docking to accommodate the ligands within the GRID values by keeping the spacing value as 0.5 A°[35–37]

Preparation of Protein

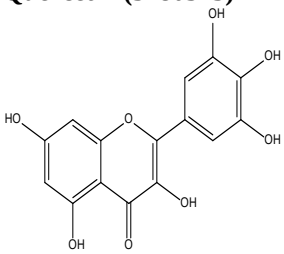
Structures of promising inflammation targets such as COX-I and COX-II are retrieved from the PDB bank with the identification numbers 6Y3C and 5KIR in SDF form and visualized in the BIOVIA Discovery software. Remove the water molecules and identify the locations of the ligand positions in the protein. Copy the attributes of the sphere created around the location of the ligand. Then, remove the ligand and save the protein in PDB file format. Convert all the ligand molecules present in individual files into PDBQT file format.



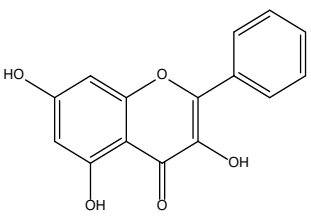
Quercetin (3280343)



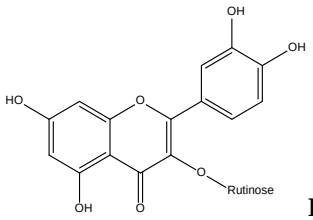
Kaempferol (5280863)



Myricetin (5281672)



Galangin (5281616)



Rutin (5280805)

Figure 2: Structure and PubChem ID of the Flavonol used in the current study

Grid generation

The prepared protein molecules are opened in the AUTODOCK VINA. Choose the protein's location and add polar hydrogen bonds and charge inducers. Set map points in the protein and paste the attributes in the grid position file by adjusting the area to 0.5 Angstroms. Give the protein and ligands a location and start the docking procedure in the command prompt.

Ligand Docking results

As a result, docking scores were saved in the file named. The same molecules in this file are visualised in discovery software to show the amino acid interactions with hydrogen and hydrophobic bonds.

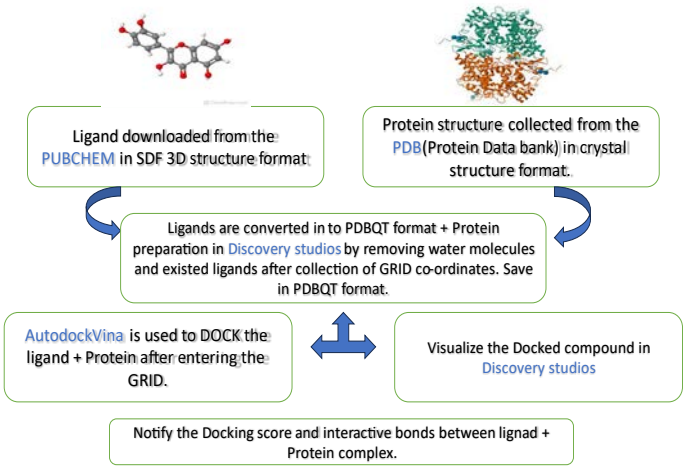


Figure 3: Molecular docking mechanism

Lipinski's rule

Lipinski's rule of five for drug-likeness is the physicochemical properties of drugs, such as Molecular weight should be less than 500Da, Hydrogen bond donor number should be less than 5, hydrogen bond acceptor number should be less than 10, and partition coefficient less than 5. As the benchmark criteria for the properties mentioned above are factors of five. So, Lipinski's rule is called the "Rule of Five". Two or more violations in these descriptors lead to No drug-likeliness.

Toxicity studies

Toxicity studies are predicated on using the pkCSM-free software to determine the regular toxic profile of the compounds mentioned above. Toxicity data includes AMES toxicity (an assay of a chemical compound's ability to induce mutations in DNA), hERG I and hERG II inhibitors, Hepatotoxicity, Skin sensitisation, and the Maximum tolerable dose of the compounds.

RESULTS

The present study is related to molecular docking between plant-derived ligands and inflammatory receptors, divided into two subclasses: 1) binding affinity and binding score estimated by Molecular docking; 2) determination of Pharmacokinetic and toxicity parameters and checking drug likeliness compatibility.

Molecular docking studies

Docking studies are computational studies that estimate the binding capacity of the ligand with the receptor. The following steps are involved in the molecular docking mechanism.

Selection of Proteins

COX-I and COX-II proteins are downloaded from the protein data bank, and their characteristics are exhibited in Table

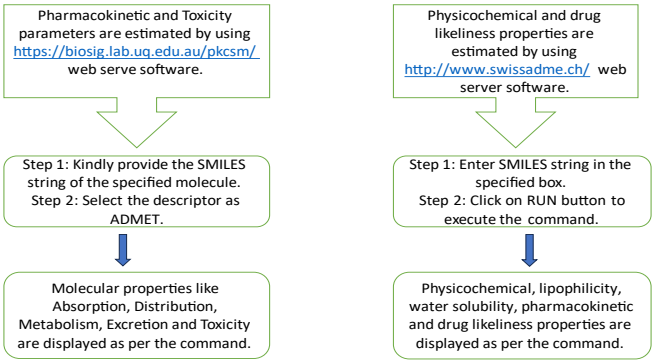


Figure 4: Pharmacokinetic properties and Drug likeliness determination mechanism by using pkCSM and SwissADME software.

Protein preparation:

After downloading the protein with the specification as mentioned above, open the protein structure with the Biovia discovery software, remove water molecules, and reduce the ligand count to one if it is bound with the multiple ligands in its structure. Define the sites of ligand and protein to edit the binding site location and save the current location of the ligand. Noted own the co-ordinates for further use. GRID coordinates are represented in Table 2. Remove the ligands from the protein site and save them in PDB file format.

Table 1: Crystallographic structures of Proteins used in study.

Property of Proteins						
Protein code	**Classification**	**Organisms**	**Resolution**	**R-Value free**	**Method**	**Chain**
6Y3C	Protein	Homosepians	3.36Å	0.263	X-RAY Diff	A
5KIR	Protein	Homosepians	2.70Å	0.22	X-RAY Diff	B

Table 2: Protein code with GRID Co-ordinates

Protein Code	**Protein**	**GRID coordinates**
6Y3C	**COX-I**	**-21.897231 -40.912692 3.444692**
5KIR	**COX-II**	**23.206000 1.318136 34.258864**

GRID Generation

In the docking procedure, the protein molecule structure is visualized in the discovery software visualiser 24.1.0.0 version to notify the molecular attachments in the ligand-receptor interaction tab. Define the ligand molecule present in the protein structure given interactions. In all the other ligand positions, water molecules are removed from their position permanently. In defining and editing the binding site, the SBD site sphere was formed around the ligand binding with protein position from the current selection tool. The current ligand site was selected to notify the sphere model and note down the attributes of the sphere in XYZ coordinates format. After selecting coordinates, remove the sphere and ligand from the protein. Save the protein for further docking studies. Save the protein and convert it into PDBQT format. Open the saved protein after conversion into PDBQT format with Autodock Vina software version 1.5.7. in the file tab, set the location preference. Open GRID tab, select macromolecule to choose protein. Set map types directly. Select Grid box to set the spacing angstrom to 0.5 Angstroms and give X, Y, Z co-ordinates.

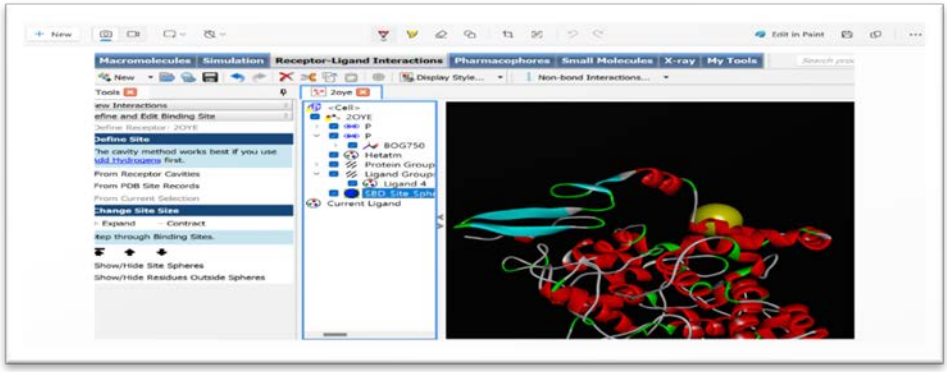


Figure 5: Grid generation by blind docking in Discovery studio visualizer.

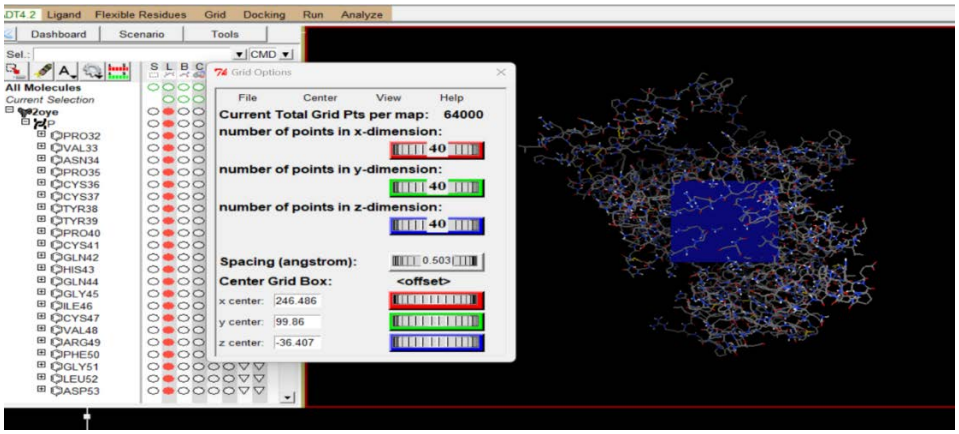


Figure 6: GRID box setting to analyze binding interaction between ligand and protein.

Docking by AutodockVina software

The converted PDBQT files are opened with AutodockVina by clicking on the directory of the software. Ad polar hydrogens and charge molecules to set up protein ready to dock. Enter the grid dimensions, set spacing angstroms 0.5A^0. Select Vina configuration and identify the protein to dock. Give the output file names as **config_multi.txt.** Open command prompt to enter the code **FOR %G IN (mol*.pdbqt) DO vina --config config_multi.txt --ligand %G --log %G_log --out result\%G_out.pdbqt** to start the Docking mechanism.

Ligands are docked with the two proteins individually and the docking scores were represented in the Table 5. Here docking to the specified proteins with the ligands and commercially available drugs also docked. Rutin registered as highest -8.7 score with COX-I protein. Galangin had a -9.4 score with COX-II protein. Rutin and Galangin had registered highest score among the other ligands and this score is more the docking score of commercially available compounds.

Ligands are bonded with the specific proteins with amino acid residues are specified in the Table 6. There are two types of bonds like hydrogen and hydrophobic bonds to interact with amino acid sequences in proteins structure. Hydrophobic bond interactions include Pi-alkyl, Pi-sigma, Pi-cation, Pi- sulfur.

Table 3: Proteins and Ligands with docking score

S. No.	Ligand	Pub chem ID	COX-1 (6Y3C)	COX-2 (5KIR)
1	Quercetin	5280343	-7.0	-9.0
2	Kaempferol	5280863	-7.0	-9.1
3	Galangin	5281616	-6.8	**-9.4**
4	Myricetin	5281672	-7.2	-8.8
5	Rutin	5280805	**-8.7**	-3.9
6	Aspirin	2244	**-5.1**	-6.3
7	Etodolac	3308	-6.8	**-7.8**
8	Indomethacin	3715	**-7.3**	-7.7

Table 4: Binding interactions (Hydrogen and Non-hydrogen) between Flavonols and inflammatory Proteins.

Protein	Ligands	Amino acid interactions	
		Hydrogen bond residues	**Non-hydrogen bond residues**
COX-I	Quercetin	HIS-43	LEU-123 (Pi -Sigma)
	Kaempferol	LYS-468, GLU-524	LEU-123 (Pi -Sigma), ARG-83(Pi-Cation)
	Galangin	THR-89, VAL-119, ARG-120	LEU-123 (Pi -Alkyl), PRO-86 (Pi -Alkyl)
	Myricetin	ARG-469	ARG-83(Pi-Cation), LEU-123 (Pi -Alkyl)
	Rutin	THR-89, PRO-84, LS-468, GLY-471, PHE-470	LEU-123 (Pi -Sigma), VAL-119 (Pi-Alkyl)
COX-II	Quercetin	GLN-192, SER-353	VAL-523 (Pi -Alkyl), VAL-349 (Pi -Alkyl), LEU-352 (Pi-Alkyl)
	Kaempferol	TYR-355, TYR-385, ILE-517, HIS-90	LEU-352 (Pi -Alkyl), VAL-523(Pi-Alkyl), VAL-349(Pi-Alkyl)
	Galangin	VAL-349, TYR-385	TRP-387 (Pi-Pi T shaped), MET-522(Pi-Sulfur)
	Myricetin	MET-522, TYR-385, HIS-90, SER-530, PHE-518, GLN-192	VAL-523 (Pi -Alkyl), LEU-352 (Pi -Alkyl), ARG-513 (Pi-Cation)
	Rutin	HIS-90, MET-522, LEU-352, TYR-355	VAL-523 (Pi -Alkyl), VAL-349(Pi-Alkyl), LEU-531, LEU-359

Molecular docking studies of the commercially available NSAIDs with inflammatory proteins

Commercially available NSAIDs are considered standard products, which can inhibit the COX-I and COX-II proteins. Etodolac is regarded as a specific COX-II inhibitor.

Pharmacokinetic and toxicity studies of flavonols

A study of the pharmacokinetic parameters is required to assess the bioavailability and kinetic behaviour of the drug particle within the body. This section divides pharmacokinetic properties into two distinct parts: the study of ADME descriptors and the toxicity profiles of the bioactive compounds.

Table 5: Binding interactions between commercially available NSAIDs and inflammatory Proteins.

Protein	Ligands	Amino acid interactions	
		Hydrogen bond residues	**Non-hydrogen bond residues**
COX-I	**Aspirin**	ARG-120	VAL-116 (Pi -Sigma), LEU-115 (Pi-Alkyl), VAL-119 (Pi-Alkyl)
	Etodolac	LYS-468, GLY-471, ARG-83	LEU-123 (Pi -Sigma), TYR-64 (Pi-Alkyl), HIS-43 (Pi-Alkyl)
	Indomethacin	ARG-79	LEU-123 (Pi -Sigma), VAL-119 (Pi-Alkyl), ARG-120 (Pi-Alkyl)
COX-II	**Aspirin**	SER-530	VAL-523 (Pi -Alkyl), LEU-352 (Pi-Alkyl)
	Etodolac	SER-530	SER-353 (Pi -Sigma), VAL-523 (Pi -Alkyl),
	Indomethacin	VAL-523, ALA-527, GLY-526, LEU-352	VAL-116 (Pi -Alkyl), TYR-355 (Pi -Alkyl), LEU-531 (Pi-Alkyl), LEU-351 (Pi-Alkyl).

ADME descriptor and Drug likeliness study

ADME (Absorption, Distribution, Metabolism, Elimination) depicts the drug molecule's kinetic behaviour, which can be measured using the SwissADME and pkCSM web server software. Kinetic descriptor studies conducted using the software are similar, as are the values they represent.

As per the Lipinski rule of five, he worked on the four properties of HBA, HBD, Molecular weight and TPSA. These properties have limits with 5 factorial values; due to this, the Lipinski rule is called the rule of five. From the above values, Rutin has the highest molecular weight and higher HBA & HBD counts. Different tools estimate the drug molecules' oral bioavailability based on the drug molecule's various kinetic properties. Ghose, Muegge, Veber, and Egan proposed drug likeliness properties. Rutin violated all the proposed limits of the pharmacokinetic descriptors values.

Toxicity profiles of the Flavonol compounds:

The estimation of toxicity profiles is used to determine the safety profile of the drug molecules. All the estimated values are mentioned in Table 10. Toxicity profiles include AMES toxicity, hERG-I and hERG-II inhibitor, hepatotoxicity, skin sensitization, and oral rat acute and chronic toxicity. The highlighted portions are represented as violating the limits of the descriptors.

Table 6: Physicochemical properties of Flavonol compounds

S. No.	Flavonol	HBA	HBD	Mol. Wt	XlogP	R. Bonds	TPSA	MR
1	Quercetin	1	5	302.33	1.5	1	127	78.03
2	Rutin	16	10	610.52	0.46	6	269.43	141.38
3	Kaempferol	6	4	286.24	1.9	1	111.13	76.01
4	Galangin	5	3	270.24	2.25	1	90.9	73.99
5	Myrcetin	8	6	318.24	1.18	1	151.59	80.06

Abbreviation: HBA-Hydrogen bond acceptor, HBD- Hydrogen bond donor, XlogP-prediction of Octonol/ water coefficient, R. Bonds- Rotational bonds, TPSA- Topological polar surface area, MR- Molar refractivity

Table 7: Drug likeliness rule prediction by various tools.

Compounds	Ghose	Muegge	Veber	Egan	Synthetic accessibility
Quercetin	Yes	Yes	Yes	Yes	3.23
Kaempferol	Yes	Yes	Yes	Yes	3.14
Galangin	Yes	Yes	Yes	Yes	3.12
Myrcetin	Yes	Yes	Yes	Yes	3.23
Rutin	**NO** (4 violations) MW>480, WLOGP<-0.4, MR>130, #atoms>70	**NO** (4 violations) MW>600, TPSA>150, H-acc>10, H-don>5	**NO** (1 violations) TPSA>140	**NO** (1 violations) TPSA>131.6	6.52

DISCUSSION

Molecular docking studies

Molecular docking studies were carried out for Flavonols to the COX-I and COX-II inflammatory proteins. Commercially available NSAIDs are considered the standard drugs for inflammation inhibition by acting against the COX proteins. Estimating binding affinity and interactive bonds between Aspirin, etodolac, and Indomethacin is the preliminary and important step in comparing the data with the test compounds.

Molecular docking of NSAIDs with COX proteins:

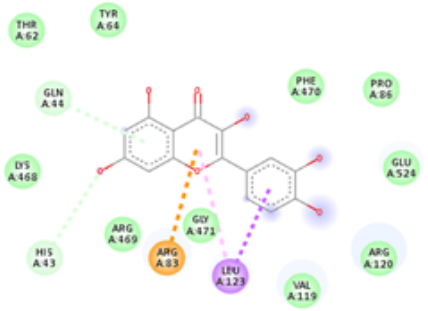
Aspirin, Etodolac, and Indomethacin are the commercially available drugs that inhibit the action of CCOX proteins. Etodolac is used to block the COX-II protein specifically; others are meant to inhibit the action of COX-I. Aspirin showed the lowest -5.1 Kcal/mol docking score in COX-I inhibition and was stabilized by the hydrogen ARG-120, Pi-sigma VAL-116 and Pi-alkyl bonds LEU-115, VAL-119. Indomethacin showed the highest binding score of -7.3 Kcal/ mol and was stabilized by the hydrogen bond ARG-79, Pi- sigma LEU-123, Pi-alkyl VAL-119, and ARG-120. The residues in indomethacin interaction are TYR A 64, ASN A80, GLY A 63, GLY A 471, and GLN A 44. In COX-II inhibition, Aspirin -6.3 Kcal/mol score was stabilised by the SER-530 hydrogen bonding and VAL-523, LEU-352 Pi-alkyl bonds. Etodolac -7.8 K cal/ mol SER-530 hydrogen bond and Pi-Sigma SER-353, Pi- alkyl VAL-523. The residues taking

part in Etodolac interaction are GLN B 192, ARG B 513, TYR B 348, HIS B 90, TYR B 355, ALA B 527, VAL B 349, GLY B 526, MET B 522, TYR B 385. Therefore, the interactions mentioned above might be significant in the inhibitory action of the COX proteins.

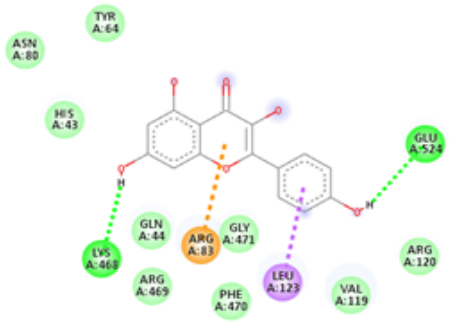
Table 8: Predicted ADME/T properties of Flavonol Compounds

Properties	**Quercetin**	**Kaempferol**	**Galangin**	**Myricetin**	**Rutin**
PSA	122.108	117.313	112.519	122.108	240.901
AlogP	1.988	2.2824	2.5768	1.988	-1.6871
Absorption					
Water Solubility (Log mol/L)	-2.925	-3.04	-3.335	-3.416	-2.892
CaCo2 Permeability (Log Papp in 10-6 cm/s)	-0.229	0.032	0.999	0.876	-0.949
Interstinal absorption (human % absorbed)	77.207	74.29	93.985	75.236	23.446
Skin Permeability (log Kp)	-2.735	-2.735	-2.735	-2.735	-2.735
P-Glycoprotein substrate	YES	YES	YES	YES	YES
P-Glycoprotein I Inhibitor	NO	NO	NO	NO	NO
P-Glycoprotein II Inhibitor	NO	NO	NO	NO	NO
Distribution					
VDss (log L/Kg)	1.559	1.274	0.816	0.106	1.663
Fraction unbound (Fu)	0.206	0.178	0.142	0.008	0.187
BBB Permeability (log BB)	-1.098	-0.939	-0.748	-1.357	-1.899
CNS Permeability (log PS)	-3.065	-2.228	-2.068	-3.467	-5.178
Metabolism					
CYP2D6 substrate	NO	NO	NO	NO	NO
CYP3A4 substrate	NO	NO	NO	NO	NO
CYP1A2 inhibitor	YES	YES	YES	YES	NO
CYP2C19 inhibitor	NO	NO	YES	NO	NO
CYP2C9 inhibitor	NO	NO	YES	NO	NO
CYP2D6 inhibitor	NO	NO	NO	NO	NO
CYP3A4 inhibitor	NO	NO	NO	NO	NO
Excretion					
Total clearance (Log ml/min/kg)	0.407	0.477	0.256	0.637	-0.369
Renal OCT2 substrate	NO	NO	NO	NO	NO
Toxicity					
AMES toxicity	NO	NO	NO	YES	NO
Max. tolerable dose		0.531	0.333	1.119	0.452
hERG I inhibitor	NO	NO	NO	NO	NO
hERG II inhibitor	NO	NO	NO	NO	YES
Hepatotoxicity	NO	NO	NO	NO	NO
Skin Sensitization	NO	NO	NO	NO	NO
Oral rat acute toxicity (LD50) mol/ kg	2.471	2.449	2.45	2.054	2.491
Oral rat Chronic toxicity (LOAEL)log mg/kg_ bw/day	2.612	2.505	2.323	3.138	3.673

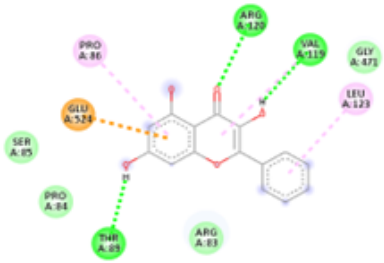
Papp- apparent permeability coefficient; AMES- assay of the ability of a chemical compound to induce mutations in DNA; Kp- Skin permeability constant; Fu- Fraction unbound; PS- Permeability surface area; LD-Lethal dose; LOAEL- Lowest observed adverse effect level; VD- volume of Distribution; hERG- Human ether a go-go- related gene



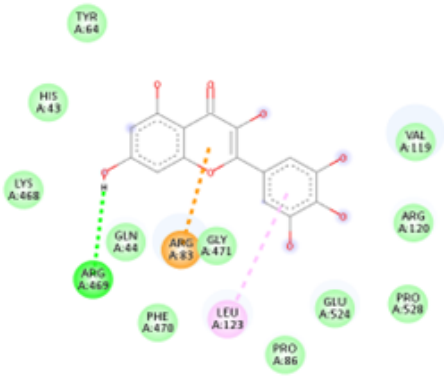
Quercetin interactions with 6Y3C



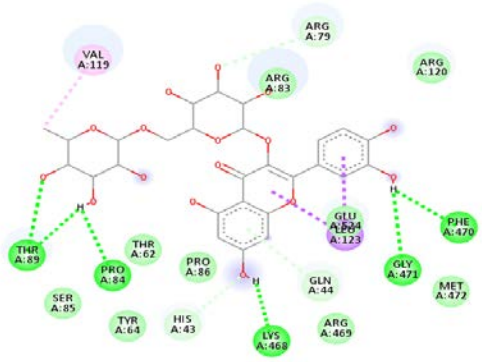
Kaempferol interactions with 6Y3C



Galangin interactions with 6Y3C

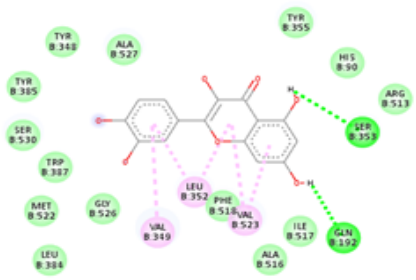


Myrcicetin interaction with 6Y3C

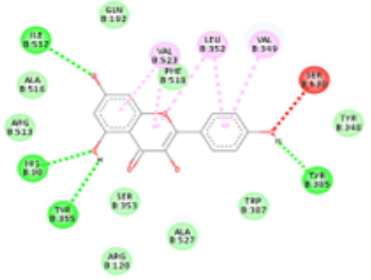


Rutin interaction with 6Y3C

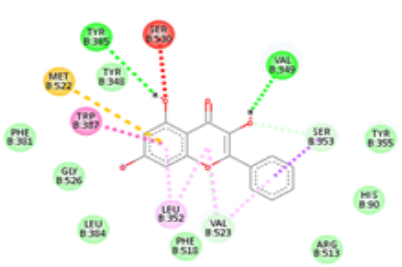
Figure 7: Schematic representation of Flavonol and COX-I (6Y3C) interactive sites elucidations.



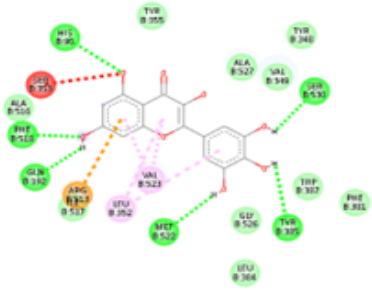
Quercetin interactions with 5KIR



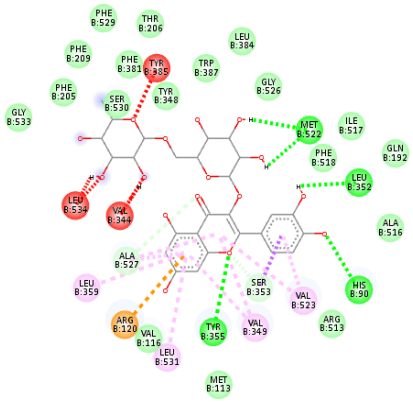
Kaempferol interactions with 5KIR



Galangin interactons with 5KIR



Myricetin interactions with 5KIR



Rutin interactions with 5KIR

Figure 8: Schematic representation of Flavonol and COX-II (5KIR) interactive sites elucidations.

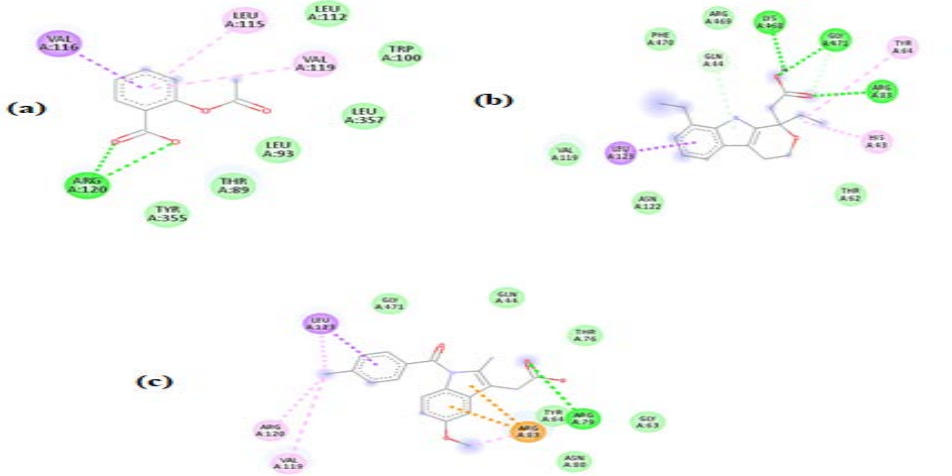
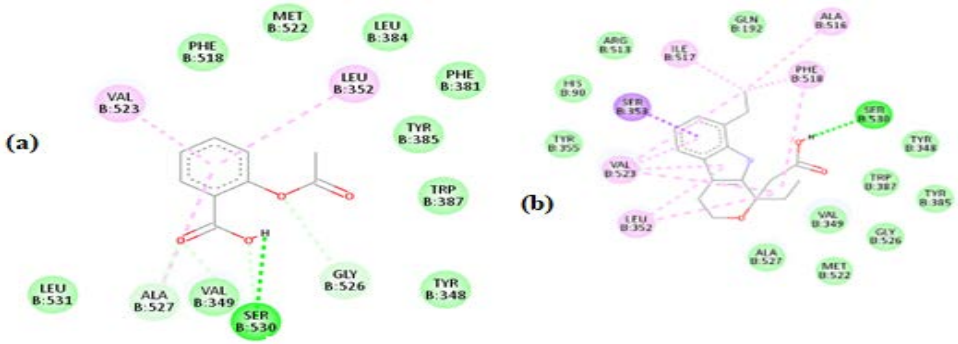


Figure 9: COX-I (6Y3C) interactive site elucidation with (a) Aspirin; (b) Etodolac; (c) Indomethacin.



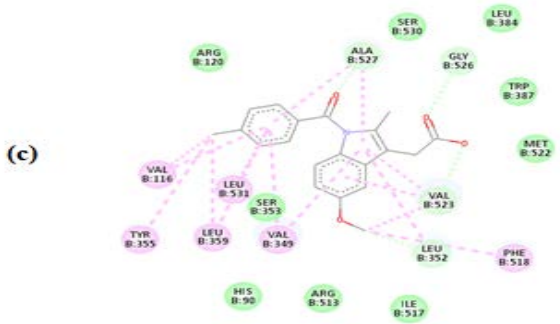


Figure 10: COX-II (5KIR) interactive site elucidation with (a) Aspirin; (b) Etodolac; (c) Indomethacin (Green dotted line: Hydrogen bonds with electronegative elements like N and O atoms; **Light green dotted line:** Carbon–hydrogen bonds; **Light purple:** Pi-alkyl interactions; **Violet dotted line:** Pi-sigma interaction; **Magenta:** Electrostatic interaction; **Green:** Van der Waals interactions)

Molecular docking of Flavonols with COX proteins:

Flavonol structures were received from the PUBCHEM data base in SDF 3D format and docked with the proteins PDB ID: 6Y3C, 5KIR. The docking mechanism was analyzed to get the better binding score which is more negative to be compared with the binding interactions of the standard drugs. This comparison is useful in the estimation of better flavonol compound with good inhibitory action. Docking positions and bonds displayed in the figure 1 & 2. Docking scores in ascending order -6.8, -7, -7, -7.2, -8.7 K cal / mol for COX-I and -3.9, -8.8, -9, -9.1, -9.4 K cal / mol for COX-II. Molecular docking studies exhibited highest docking score for COX-I is Rutin (PUB CHEM ID: 5280805) -8.7 K cal / mol and hydrogen bond with THR-89, PRO-84, LS-468, GLY-471, PHE-470, Pi- Sigma bonding LEU-123, Pi- alkyl bonding VAL-119. Highest docking for COX-II is Galangin (PUB CHEM ID: 5281616) -9.4 K cal / mol and hydrogen bonding with VAL-349, TYR-385, Pi- sulfur MET-522, Pi-Pi t shaped TRP B 387. In this Pi-Pi interactions are formed due to the orientation of aromatic ring structures and electron cloud sharing between those rings. Pi-Pi t shaped bonds are like orientation of ring structures in T shaped structure. Pi-sulfur bond formed due to sulfur compound present in the amino acid residues[33,34].

Interactions between ligand and protein were identified by the AutodockVina and biovia discovery visualizer softwares. Covalent hydrogen bonding of ligand molecule observed between carbonyl O, hydroxyl O to the amino and carbonyl group of amino acids. Pi-Pi interactions are observed between the phenyl ring of ligand to the phenyl ring of VAL-523, VAL-352. Pi- cation interaction with ARG-83 amino acid observed to loss the electron from the amino group of ARG-83 to become cation. The molecular docking studies revealed the similar binding between bioactive ligand and standard drug products. In docking images green colour dotted line indicates hydrogen bond, a violet colour dotted line represents Pi- sigma bonds, Pi-alkyl indicated with light purple colour, Pi-cation indicated with orange colour bonds, Pi- Pi t shaped indicates with light pink colour.

Comparative studies between Docking scores of NSAID's and Flavonols.

Rutin showed the maximum docking score with respective to the COX-I receptor in inflammation mechanism. When it is compared with the Aspirin, hydrogen bonding is very less in number count i.e. THR-89, PRO-84, LS-468, GLY-471, PHE-470 are the hydrogen bonding present between COX-I and Rutin. Aspirin and COX-I has only one hydrogen bond like ARG-120. Other bonding like non-hydrogen bonding is Pi-Alkyl, Pi-Sigma and the Vander wall forces.

ADME/T studies:

ADME studies are performed to know the drug likeliness and pharmacokinetic nature of the flavonols. after molecular docking flavonol compounds are subjected to drug likeliness with SwissADME software, toxicity studies by pkCSM web servers. Newly invented drug molecule for delivery into the body cavities, it should obey the Lipinski's rule of five stated as MW≤ 500, HBA ≤ 10, HBD ≤ 5, and log P ≤ 5. As per the Ghose rule of drug likeliness it should have molecular weight within the range of 160-480, logP 0.4 – 5.6, atom count 20 to 70, molar refractivity 40-130, TPSA < 140. The muegge rule states that

MW<600, TPSA< 150, Hydrogen acceptors < 10, and Hydrogen donors < 5. Veber rule: TPSA ≤ 160, number of rotatable bonds ≤ 10. Egan rule; TPSA ≤ 132, logP -1 to 6. Apart from all the bioactive compounds, Rutin violated all the drug-likeliness properties. Lipophilicity (partition coefficient) should be less than 5 for better absorption. In drug development, the molecular weight and lipophilicity are modified to improve the affinity and selectivity of the drug candidate[35]. The docking hits have lower TPSA except for rutin (the addition of glucose in the structure may modify the properties); the oral bioavailability is inversely proposed to the topological polar surface area[36]. Galangin is having high oral bioavailability[37–39].

Table 9: This table presents a clear comparison between NSAIDs and flavonols across different key aspects, helping to evaluate the therapeutic potential of flavonols in contrast to traditional NSAIDs [41]

Aspect	NSAID's	Flavonol
Docking Scores & Binding Affinity	Strong binding to COX-I and COX-II enzymes, high efficacy in inhibition.	May exhibit weaker binding affinity, docking scores could be lower.
Mechanism of Action	Block COX enzymes' active sites, preventing arachidonic acid conversion to prostaglandins (reversible or irreversible).	May involve indirect inhibition (e.g., enzyme conformation modulation, antioxidant effects).
Bioavailability & Efficacy	Designed for optimized bioavailability, high absorption rates and sustained activity.	May have lower absorption, poor bioavailability, requiring higher doses or formulation optimization.
Selectivity for COX-I vs. COX-II	Non-selective (both COX-I and COX-II) or COX-2 selective (e.g., celecoxib)	Potential for selective COX-II inhibition, may reduce gastrointestinal side effects

In conclusion, structural differences such as functional group orientation, molecular rigidity, hydrophobicity, and steric hindrance likely contribute to the observed differences in binding affinity. These factors, coupled with statistical analysis of binding energies, can provide a more comprehensive explanation of the interaction differences between flavonols and NSAIDs.

In-depth predictions of the pharmacokinetic properties are studied in detail in Table 10. Basic properties like absorption and lipophilicity are essential to reach the drug molecule at the absorption site. PSA should be <140 & AlogP should be < 5 as per the specification to show ideal solubility and permeability. Absorption characteristics like Papp, intestinal absorption, skin permeability, Pgp substrate and inhibition. papp permeability value should be >0.90 to exhibit good permeability, intestinal absorption must be > 30%, and Skin permeability >-2.5 is considered low skin permeation. P-glycoprotein substrate and inhibition are related to the exuded nature of the protein to the outside molecules. Quercetin and Kaempferol showed less permeation, and rutin has lesser intestinal absorption. All the compounds have an affinity to bind with the Pgp protein molecules. The volume of distribution parameters like VDss, Fraction unbound, BB permeation and CNS permeability showed better results. VD < -0.15 has less distribution, >0.3 of BBB indicates easily crosses the brain barrier, <-3 easily penetrates the CNS. only kaempferol, galangin can cross the CNS and No drug crosses the BB barrier. CYP450 is the enzyme related to the metabolism of the drugs in the liver. all four compounds except rutin can inhibit CYP1A2, and galanin can bind to the inhibitors of CYP1A2, CYP2C19 and CYP2C9. All the compounds have less clearance rate from the body, and it can depend on the drug's molecular weight and hydrophilicity nature.

Drug likeliness properties are the key parameters to report the possibility of oral drug delivery in the compounds by studying properties like molecular weight, lipid solubility, hydrogen bond donors, hydrogen bond acceptors, and rotatable bonds. Violation of more than one property may lead to cause bioavailability problems. A toxicological properties study may indicate the toxic effects of the drug, and the hERG II inhibitor action shown by the rutin may cause it. AMES toxicity related to the induced mutations in genes, hepatotoxicity and skin sensitisation was reported as having no implications. In the context of ADME/T (Absorption, Distribution, Metabolism, Excretion, and Toxicity) analysis, the study adheres to Lipinski's Rule of Five, which is a fundamental guideline for evaluating the drug-likeness of compounds. Lipinski's Rule assesses properties such as molecular weight, lipophilicity, hydrogen bond donors and acceptors, and rotatable bonds, all critical factors influencing oral bioavailability. Adhering to these rules suggests that the compounds under investigation have favourable pharmacokinetic profiles. In addition to Lipinski's Rule of Five, the study incorporates other drug-likeness criteria from tools like

Ghose and Muegge. These tools provide additional insights into the chemical space and help identify potential candidates with properties conducive to successful drug development. Ghose's criteria focus on molecular properties, including size, polar surface area, and the number of rotatable bonds. At the same time, Muegge's metrics consider more specific aspects of drug behaviour, such as water solubility and absorption potential. Together, these methods offer a more comprehensive understanding of the compound's drug-like characteristics, aiding in selecting promising candidates.

However, one area that could be expanded upon is the discussion of toxicological implications. While the ADME/T analysis focuses on the pharmacokinetics and physicochemical properties of the compounds, understanding their potential toxicity is essential for comprehensive drug development. A more detailed exploration of toxicological factors, such as cytotoxicity, mutagenicity, carcinogenicity, and organ-specific toxicity, would further support the safety profile of the compounds. Additionally, predictive tools like QSAR (Quantitative Structure-Activity Relationship) models or computational toxicology platforms could identify compounds with potential adverse effects, guiding the optimisation process to reduce toxicity while enhancing efficacy. Toxicity studies are essential to know the safety of the compounds. all the compounds have no hepatotoxicity and no skin sensitisation. Rutin has the hERG -II inhibitor effect to produce cardiotoxicity. Myricetin has the AMES toxicity to initiate the changes in DNA.

Structural modification suggestions for improving the Drug likeliness of Rutin:

Table 10: Summary of the recommendations for improving the pharmacokinetic properties of Rutin in table format [47,48]

Modification Strategy	Effect	Explanation
Increase Lipophilicity (for better membrane penetration)	Improve absorption through the GI tract, especially in oral administration.	By incorporating hydrophobic groups (alkyl or aryl), Rutin's ability to penetrate cellular membranes can be enhanced, increasing bioavailability.
Improve Solubility (for better dissolution and absorption)	Enhance Rutin's dissolution in the gastrointestinal tract, leading to better absorption.	Adding functional groups like hydroxyl groups or salts can improve water solubility, facilitating better absorption, particularly in oral formulations.
Alter the Glycoside Structure.	Improve bioavailability by making Rutin more permeable.	Modifying the sugar portion of Rutin, removing it, or attaching it to a more lipophilic structure could enhance permeability and membrane crossing.
Prodrug Strategy	Enhance pharmacokinetics by optimizing absorption and reducing first-pass metabolism.	Converting Rutin into a prodrug form allows for better absorption by temporarily masking polar groups before the prodrug is metabolized into the active form.

Toxicity of Flavonoids: hERG Inhibition and Cardiovascular Risks

Flavonoids, such as rutin, have garnered attention for their potential therapeutic properties, including antioxidant, anti-inflammatory, and anticancer effects. However, recent studies have highlighted potential cardiovascular toxicity associated with inhibiting the human Ether-à-go-go-Related Gene (hERG) potassium channel. The hERG channel is critical in regulating cardiac action potentials, and its inhibition can lead to arrhythmias, prolonged QT intervals, and an increased risk of torsades de pointes, a potentially fatal arrhythmia.[40,41] Rutin, a flavonoid found in various plants, has been identified as a compound capable of inhibiting hERG channels in in vitro studies. This presents a potential concern for its clinical application, especially in patients with pre-existing heart conditions or those taking medications targeting cardiac ion channels.

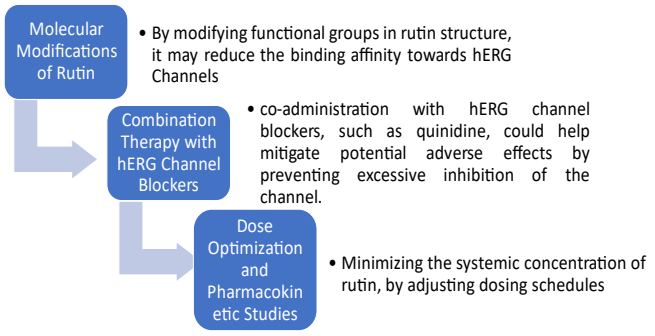


Figure 11: Possible Pathways for Mitigating Adverse Cardiovascular Effects.

While rutin's hERG inhibition is a valid concern, it does not necessarily preclude its use as a therapeutic agent. Further

research into molecular modifications, delivery systems, and safety monitoring could mitigate the adverse cardiovascular effects associated with hERG inhibition, paving the way for safer clinical applications of flavonoids like rutin.

Table 11: Comparative studies of NSAIDs and Flavonols ADME/T properties.

Parameter	Flavonols	NSAIDs
Absorption	Well absorbed in the gastrointestinal tract but may have low bioavailability due to first-pass metabolism.	Typically, it is well absorbed in the stomach and small intestine.
Distribution	Distributed widely in tissues, especially in the liver, kidneys, and brain. Can cross the BBB.	Widely distributed, especially in plasma, tissues, and synovial fluid. Can cross the blood-brain barrier, depending on the NSAID.
Metabolism	Primarily metabolized by phase I enzymes (CYP450), producing conjugates.	Metabolized mainly by phase I and phase II enzymes (CYP450, UDP-glucuronosyltransferase). Some NSAIDs have active metabolites.
Excretion	Excreted primarily through urine after phase II metabolism (conjugates).	Mainly excreted by the kidneys as metabolites or unchanged.
Toxicity	Generally well-tolerated, with rare reports of liver toxicity or allergic reactions. High doses may lead to gastrointestinal disturbances.	Potential for gastrointestinal ulcers, renal impairment, and cardiovascular risks, especially with chronic use. Hepatic toxicity in some cases.
Bioavailability	Low bioavailability due to extensive first-pass metabolism and poor solubility.	Generally moderate to high bioavailability, but affected by formulation and food intake.

Based on the standard journal procedures, flavonols are evaluated for the ADME/T and docking studies to exhibit the docking scores of the NSAIDs and Flavonol compounds. Docking studies were performed by AUTODOCK VINA software and BIOVIA DISCOVERY software, which were used to prepare the protein molecules before docking studies. pkCSM and SWISSADME were used to evaluate the ADME and Toxicity properties. These are well-known, unique software to notify the kinetic and dynamic properties of the drug molecules. All flavonols included in the study were evaluated under identical conditions for the docking, ADME (Absorption, Distribution, Metabolism, Excretion), and toxicity tests. The docking simulations were performed using the same parameters and software, ensuring consistency across all compounds. Similarly, ADME predictions and toxicity assessments used the same methods and algorithms to provide reliable and comparable results.

CONCLUSION

The polyhydroxy group-containing naturally occurring dietary flavonoid compounds known as flavonols have a binding affinity for COX-I and COX-II inhibition, which indicates the bioactive compounds' anti-inflammatory activity. The current study sought to demonstrate the anti-inflammatory impact and found that, compared to NSAIDs sold commercially, these flavonols had a greater ability to inhibit COX proteins with fewer adverse effects. ADME characteristics show the potential for oral bioavailability, absorption, and penetration mechanisms. Studies on toxicity indicate that it is not mutagenic or carcinogenic.

We can create pharmaceutically effective medications using these natural substances in an appropriate dose form with the help of this data. More wet lab research is needed to reach the human health safety profile, and comprehensive drug interaction investigations must be conducted. One of the processes behind several autoimmune illnesses, including psoriasis and rheumatoid arthritis, is anti-inflammatory activity. Taking the natural ingredients in an appropriate medication delivery dose can lessen the financial load and minimise the adverse effects of autoimmune illnesses.

FINANCIAL ASSISTANCE

NIL

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTION

All authors contributed significantly to conception, methodology, data analysis, and writing. Narendra Pentu took

part in drafting and analysing for intellectual content. Ajitha Azhakesan and Pasupuleti Kishore Kumar contributed to software validation, visualisation, interpretation, supervision, review and editing.

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