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Research Article

MODELING OF COX-2 INHIBOTORY ACTIVITY OF FLAVONOIDS

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ABSTRACT

Flavonoids are a widely distributed group of natural products with a variety of pharmacological activities including antioxidant, anticancer and anti-inflammatory activities. There are a variety of mechanisms reported to explain the anti-inflammatory activity of flavonoids. The present work involves use of in silico methods to study the binding of flavonoids to COX-2. The flavonoids involved in the present work were members of flavonol, flavone, flavanone or isoflavone class. Docking studies were carried out to determine whether flavonoids can act as COX-2 inhibitors. The results indicated that some flavonols and flavones containing a 2,3-double bond may act as preferential inhibitors of COX-2.

Keywords: Cyclooxygenase(COX), Flavonoids, Anti-inflammatory activity, Docking studies, Structure Activity Relationship.

INTRODUCTION

Cyclooxygenase (COX) is an endogenous enzyme which catalyses the conversion of arachidonic acid into Prostaglandins and thromboxanes.[1,2] The enzyme exists in atleast two isoforms, COX-1 and COX-2. Although both the isoforms catalyze the same biochemical transformation, the two isoforms are subject to a different expression regulation.[3] COX-1 is a constitutive enzyme and is responsible for the supply of prostaglandins which maintain the integrity of the gastric mucosa and provide adequate vascular homeostasis whereas COX-2 is an inducible enzyme and is expressed only after an inflammatory stimulus.[4,5] The function of COX-2 is to synthesize prostaglandins for the induction of the inflammation[6] and pain. This discovery led to the development of selective COX-2 inhibitors which are a class if compounds with good anti-inflammatory activity and reduced gastrointestinal side effects.[7-9] Over expression of COX-2 has also been demonstrated in various human malignancies[10] and inhibitors of COX-2 have been shown to reduce the risk of gastrointestinal, skin, breast and bladder tumors.[11-13] While the mechanism of action is not completely defined, the over expression of COX-2 has been shown to inhibit apoptosis and increase the invasiveness of tumorgenic cell types.[14]

Flavonoids are a widely distributed group of natural products with a variety of pharmacological activities including antioxidant, anticancer and anti-inflammatory activities.[15,16] The anti-inflammatory activity is reported due to inhibition of lipooxygenase by the flavonoids. Flavonoids are also known to suppress COX-2 transcription.[17] Literature reports that the anti-inflammatory and the anticancer activities of the polyphenols may be related to each other in view of the overexpression of COX-2 in some types of cancers. However there is no conclusive report as to whether the anti-inflammatory activity of the flavonoids is due to inhibition of enzymes LOX, COX-1, COX-2 or due to inhibition of transcription of COX-2. In view of these literature findings, we decided to dock a series of flavonoids into the active site of COX-2 and study the binding pattern.

MATERIALS AND METHODS

The Docking studies were carried out by using GLIDE (Maestro, version 8.5, Schrödinger, LLC, 2008) software. The crystal structure of the enzyme COX-2 complexed with SC-558 was obtained from Protein Data Bank (PDB code: 1CX2) and was used for the docking studies. The enzyme exists in a tetrameric form in the crystal. The water molecules in the crystal were not considered in docking as none of them were found conserved within the binding zone of the ligand in the crystal structures. The crystal structure was 'cleaned' by deleting the ligand and the cofactors. This was followed by adding hydrogen atoms in their standard geometry, adjusting the bond orders and formal charges. The crystal structure was then refined and the geometries were optimized with the OPLS_2005 forcefield using standard protocol and parameters as included in GLIDE. The ligand structures were built, manipulated and adjusted for chemical correctness using Maestro 7.5 (Maestro, v7.5, Schrödinger LLC) graphical user interface employing Macromodel 9.5 (Macromodel, v9.5, Schrödinger LLC). The ligands were geometry minimized using the OPLS_2005 force field and the Truncated Newton Conjugate Gradient to a gradient RMSD below 0.01 kJ/Å.

Receptor Grid Generation

A grid was generated using the information on the crystal structure and docking information on the synthetic COX-1 and COX-2 inhibitors previously reported in the literature. Thus the active site comprised of His^{90}, Arg^{120}, Tyr^{355}, Tyr^{385}, Arg^{513}, Val^{523} and Ser^{530}.[18,19]

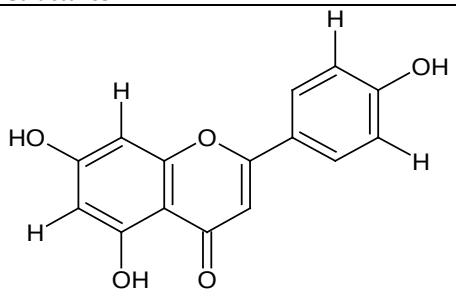
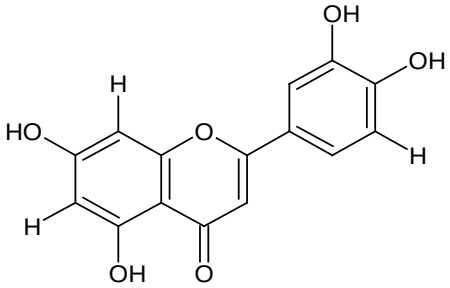
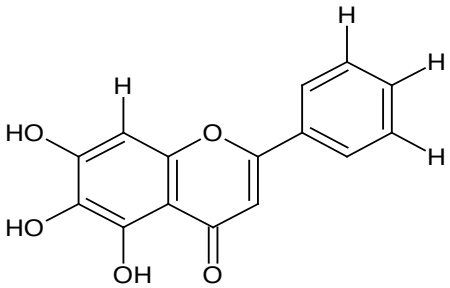
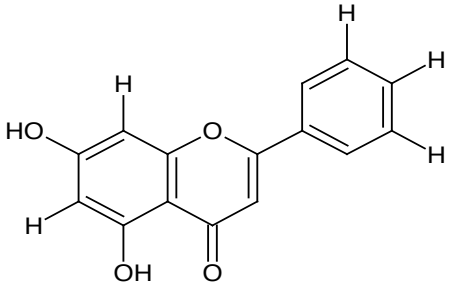
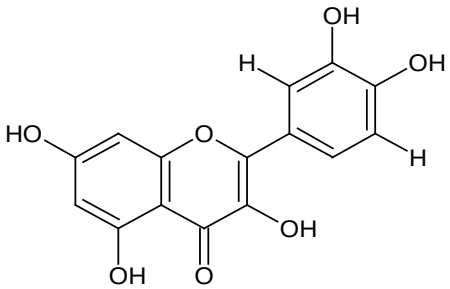
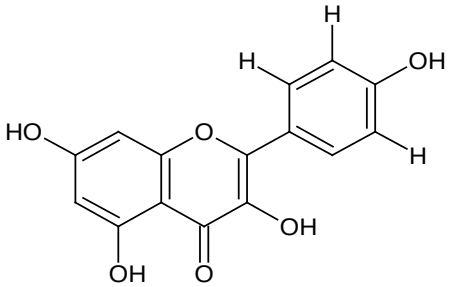
Ligand Docking

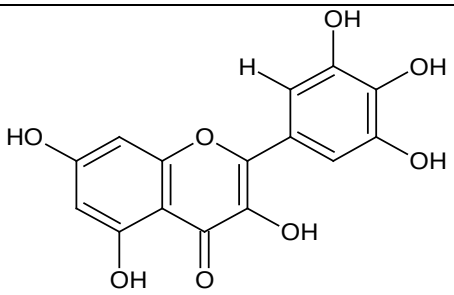
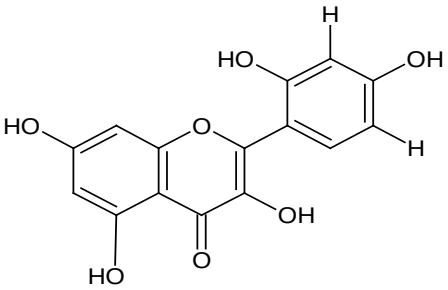
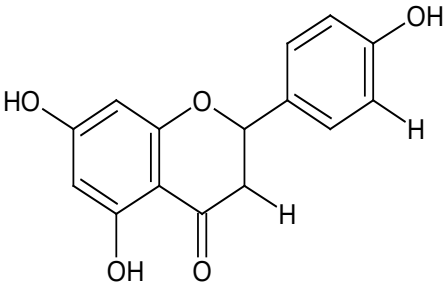
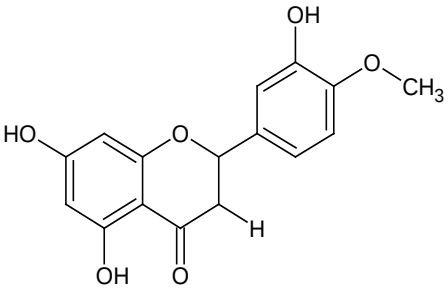
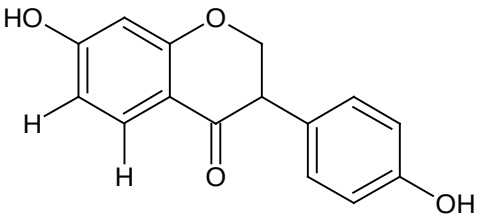
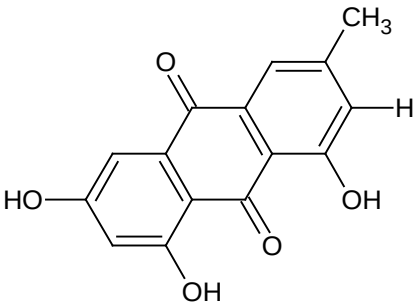
SC-558 was initially docked into the active site of the enzyme using the extra-precision mode. During the docking procedure, ligand was flexible whereas the receptor was held rigid. The best docked pose was saved. The rmsd between the crystal structure and the docked pose was 0.63, thereby validating the docking protocol. The ligands selected for the present study consisted of two types, viz. Synthetic inhibitors of COX-2 and flavonoids. The structures of the 9 synthetic inhibitors of COX-2 along with their reported pIC_{50} values and obtained G-scores have been shown in Table I. The dataset of flavonoids and G-scores of flavonoids have shown in Table II and Table III respectively. Both the groups of ligands were docked into the active site of COX-2. Fig. I-II indicates the docked images of SC-558 and Diclofenac. Fig. III-VI indicates the docked images of flavonoids.

Table I: Synthetic COX-2 inhibitors with their pIC_{50} (-log IC_{50}) and G_Score

S.No	Compound Name	pIC_{50}	G_Score
1.	Terphenyls	2.301	10.225
2.	SC-558	2.0458	10.236
3.	Flosulide	1.8239	8.63
4.	Celecoxib	1.3979	9.736
5.	L 745,337	1.301	9.171
6.	Pyrrols 2	1.301	8.541
7.	Rofecoxib	1.1308	7.433
8.	SC-58125	1.0458	8.714
9.	Zomeplrac	-0.301	4.921

Table II: Dataset of flavonoids

Serial No.	Compounds Name	Structures
1	Apigenin	
2	Luteolin	
3	Baicalein	
4	Chrysin	
5	Quercetin	
6	Kaemferol	

7	Myricetin	
8	Morin	
9	Naringenin	
10	Hesperetin	
11	Genistein	
12	Emodin	

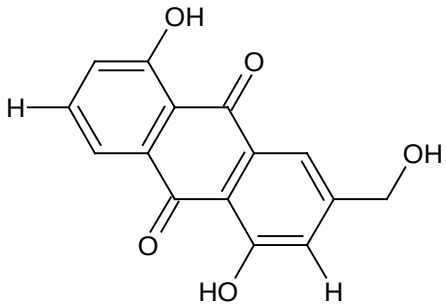
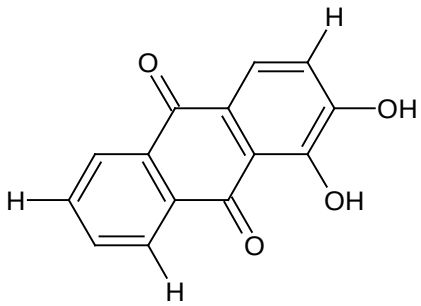
13	Aloe-emodin	
14	Alizarin	

Table III: Flavonoids with their G_Score

Sr. No.	Ligand Name	G_Score
1.	Myrecitin	-10.3199
2.	Baicalein	-10.2358
3.	Luteolin	-8.7504
4.	Chrysin	-8.3348
5.	Apigenin	-8.2802
6.	Quercetin	-8.1414
7.	Genistein	-7.0115
8.	Hesperetin	-6.3133
9.	Morin	-6.2224
10.	Aloe-emodin	-6.1079
11.	Kaempferol	-6.0199
12.	Alizarin	-5.7319
13.	Naringenin	-5.2815
14.	Emodin	-5.2342

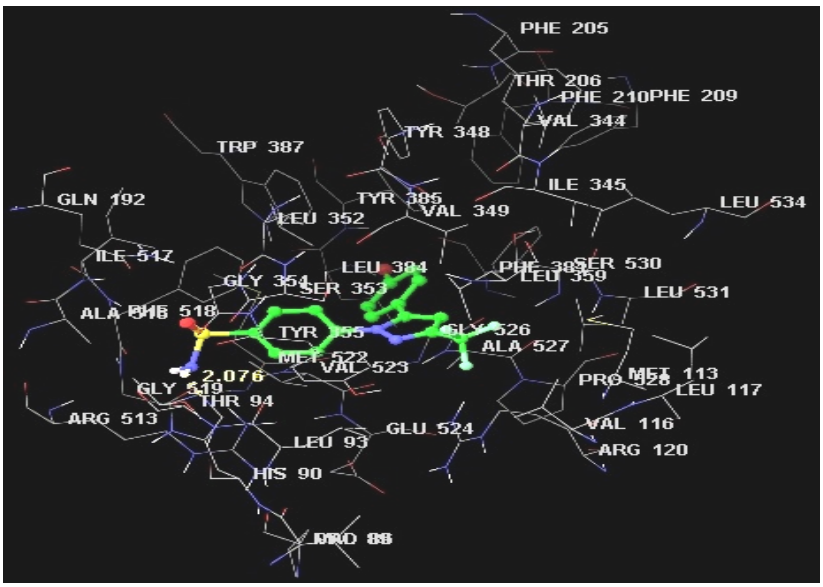


Fig. I: Docked Images of Synthetic compounds on COX-2: SC-558

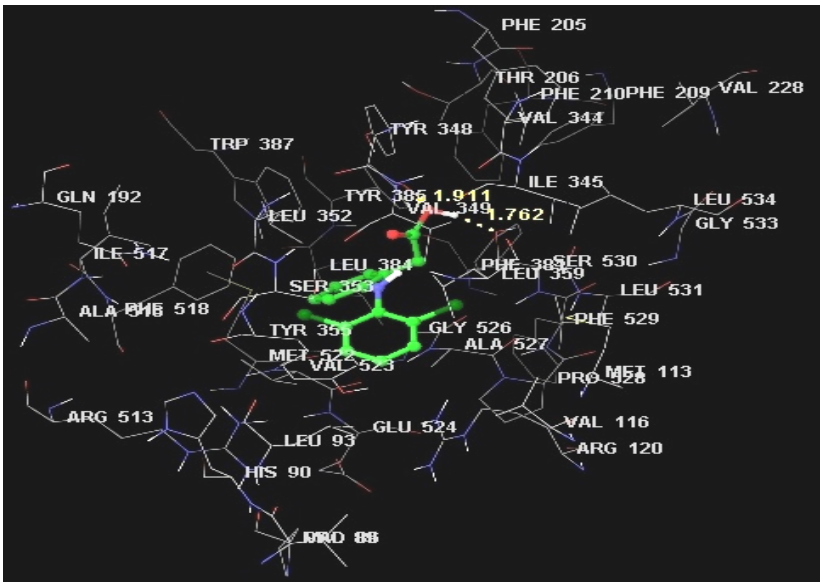


Fig. II: Docked Images of Synthetic compounds on COX-2: Diclofenac

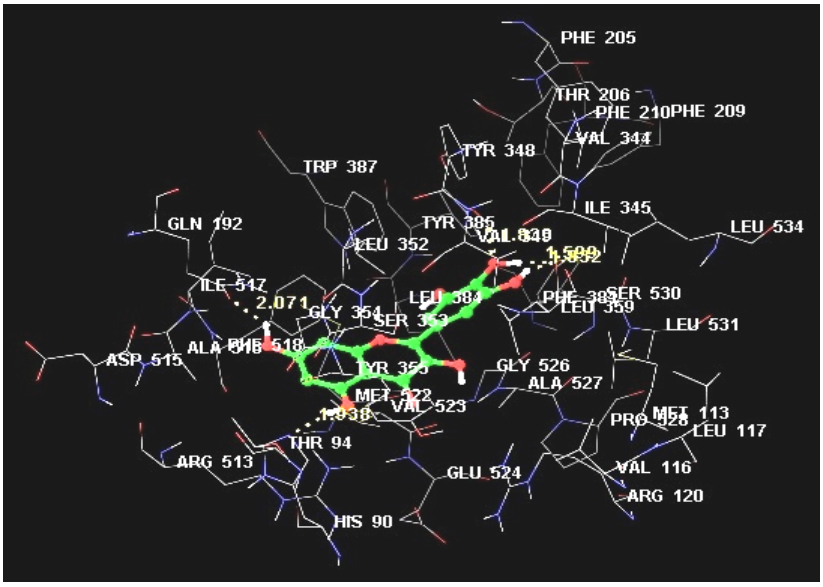


Fig. III: Docked Images of Flavonoids on COX-2: Myrecitin

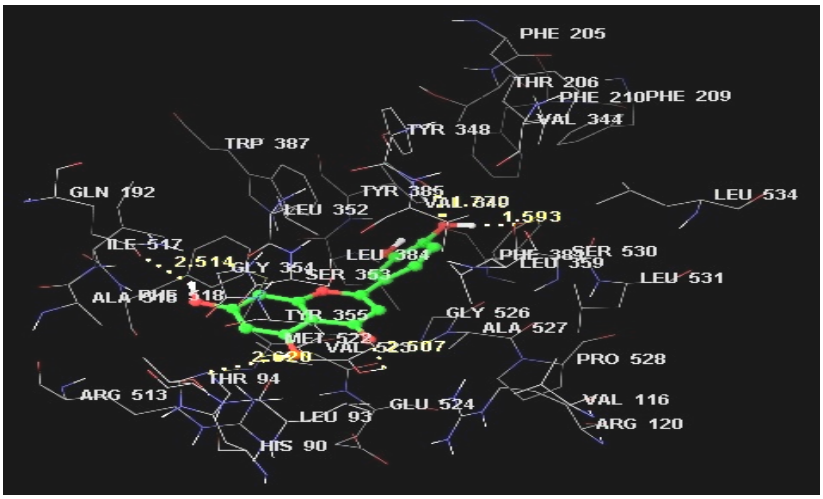


Fig. IV: Docked Images of Flavonoids on COX-2: Luteolin

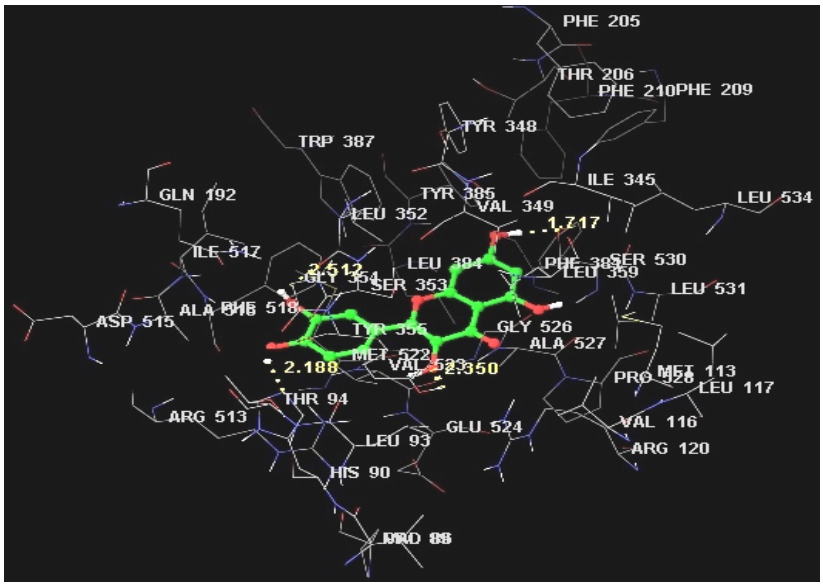


Fig. V: Docked Images of Flavonoids on COX-2: Quercetin

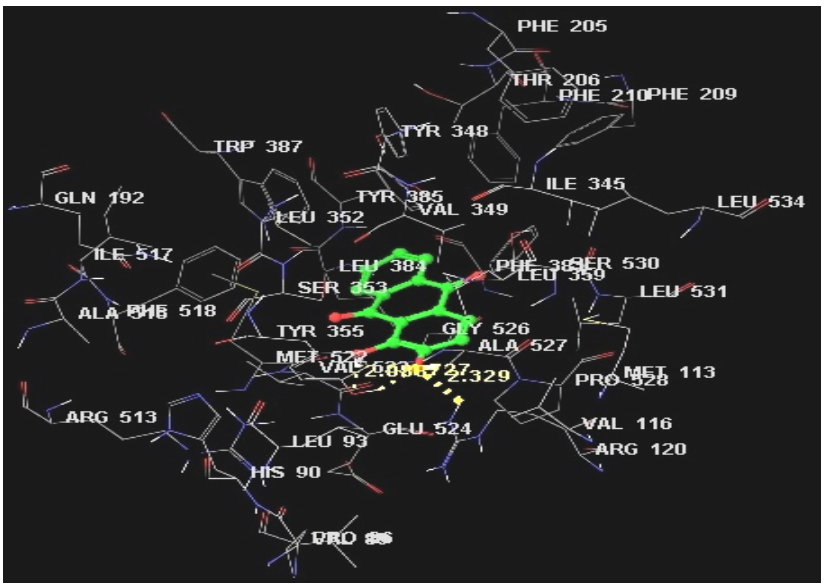


Fig. VI: Docked Images of Flavonoids on COX-2: Alizarin

RESULTS AND DISCUSSION

The active site of COX-2 is divided into three important regions[20], the first being a hydrophobic pocket defined by Tyr^{385}, Trp^{387}, Phe^{518}, Ala^{201}, Tyr^{248} and Leu^{352}; the second region being the entrance of the active site lined with the hydrophilic residues Arg^{120}, Glu^{524}, Tyr^{355} and the third is a side pocket lined by His^{90} Arg^{513} and Val^{523} Docking of the synthetic compounds indicated that these compounds show three different types of binding patterns. In case of the selective COX-2 inhibitors such as SC-558, the phenyl ring was in the close vicinity of the hydrophobic pocket and the phenylsulphonamide group occupied the side pocket and showed binding with His^{90} and an interaction with Arg^{513} which has also been identified as an important residue in the binding of selective COX-2 inhibitors according to the site-directed mutagenesis data. The results are in accordance with the literature reports of docking of the selective COX-2 inhibitors.[20] Docking of Diclofenac revealed that its orientation makes the residues of the side pocket and the hydrophilic pocket inaccessible. The phenyl acetic acid moiety is orientated towards Tyr^{385} and Ser^{530} and shows H-bonding interaction. When compounds like Ibuprofen and Naproxen were docked into the active site of the enzyme, the interacting residues were found to be Arg^{120} and Tyr^{355}. These results are consistent with those reported in the literature.[20]

Initially, 11 flavonoids belonging to the chemical classes of flavonol, flavone, flavanone and isoflavone were docked into the active site of COX-2. Analysis of the docked poses of these compounds showed that flavonoids too, show different types of binding patterns as in case of synthetic NSAIDs. Flavonols such as Myrecitin and Quercetin and flavones Baicalein and Luteolin show the presence of catechol moiety in their structure. Myrecitin and Luteolin contain the catechol function on the B-ring which is orientated towards the hydrophobic pocket, with 3',4'-dihydroxy groups forming H-bonds with Tyr^{385} and Ser^{530}. Myricetin also showed an additional interaction with His^{90} However, the Arg^{513}, which has been named as an important residue involved in the binding of selective COX-2 inhibitors, remained inaccessible leading to the conclusion that Myricetin can not act as a selective COX-2 inhibitor. Baicalin contains the catechol-like function on the A-ring, which is orientated towards the hydrophobic pocket, forming H-bonds with Tyr^{385} and Ser^{530} Importance of the catechol-like function was further confirmed by

docking 7,8-dihydroxyflavone into the active site of COX-2, whereupon the 7,8-dihydroxy groups interacted with Tyr^{385} and Ser^{530} and also gave a good G-score. These compounds therefore are proposed to inhibit both COX-1 and COX-2 enzymes. Although Quercetin contains a catechol-like function on the B-ring like Myrecitin and Luteolin, the B-ring was not orientated towards the hydrophobic pocket. Instead, the A-ring was orientated towards the pocket, forming H-bond with Ser^{530} Absence of another OH group however prevented interaction with Tyr^{385} but the orientation enabled the 3-OH group to interact with Tyr^{355} This may lead to a conclusion that Quercetin may not be a classical COX inhibitor but may act as anti-inflammatory agent via a different pathway which probably involves inhibition of transcription of COX-2.

The flavonoids of the dataset devoid of the catechol function included flavones Apigenin and Chrysin, flavonols morin and Kaempferol, flavanones Naringenin and Hesperitin and isoflavone Genistein. When we docked the flavonols and flavones devoid of the catechol-like moiety, the binding pattern shifted from involvement of Tyr^{385} and Ser^{530} to interaction with Arg^{120} and/or $Tyr.^{355}$ When Chrysin and Apigenin were docked into the active site of COX-2, the 5-OH interacted with Tyr^{355} forming a H-bond. The 4'-OH on the B-ring of Apigenin formed an additional H-bond with Tyr^{385} However Ser^{530} remained inaccessible, thereby underlining the importance of the catechol moiety. Results for Morin and Kaempferol indicated an interaction with Arg^{120} and Tyr^{355} along with low G-scores.These two compounds may act as weak COX-1 inhibitors without any significant COX-2 inhibition. The orientation of Hesperitin and Naringenin in the active site of COX-2 was such that in spite of the catechol-like function being present, an interaction with Tyr^{385} and Ser^{530} is not seen. Instead, the 7-OH of the A-ring interacted with Arg^{120} This may be due to the absence of the double bond which changes the geometry of the C-2 such that it orients the A-ring towards the Arg^{120} The interaction with Tyr^{355} was also not seen leading to very unfavourable G-scores associated with these compounds. These compounds probably do not inhibit the enzyme COX.

Experimental results reported in the literature on the COX-2 inhibitory activity of the flavonoids are not consistent. The docking results obtained in the present study were compared with the literature reported COX-1 and COX-2 inhibitory activities of some of the flavonoids. Baicalin Chrysin and 7,8-dihydroxyflavone are reported to inhibit both COX-1 and COX-2.[23] Quercetin is reported to be a very weak inhibitor of COX-1 as well as COX-2 but possessing a fairly good anti-inflammatory activity due to its ability to inhibit phospholipase A_2 and to down-regulate COX-2 expression.[24] Kaempferol[21] is reported to be an inhibitor of the COX-1 catalyzed PG biosynthesis, with an IC_{50} value of 7.5 μM, whereas Naringenin[21] is reported to possess a very weak COX-1 inhibitory activity. These results are fairly in accordance with our docking results

All the flavonoids included in this study are reported to possess anticancer activity. Since these compounds exhibited good binding to COX-2 *in silico*, we decided to dock naturally occurring 9,10-anthraquinones including emodin, aloe-emodin and alizarin. These compounds are also reported to possess anticancer activity, however because of the presence of anthraquinone moiety, the structure is more planar. The results of docking indicated that these compounds do not show appreciable binding to COX-2 *in silico*. These results are also consistent with the reports[20] that synthetic compounds with planar geometry show less efficient ligand-receptor interactions at COX-2.

COX-2 which is overexpressed in a variety of tumors, has been indicated to play an important role in the carcinogenesis. Overexpression of COX-2 inhibits apoptosis and increases cancer cell proliferation and tumor angiogenesis.[22] Flavonoids with anticancer activity may therefore act via multiple mechanisms involving inhibition of COX, inhibition of LOX, inhibition of COX transcription and other mechanisms which lead to decreased levels of inflammatory prostaglandins.

CONCLUSION

The COX-2 inhibitory activity of some flavonoids has been modeled. The flavonoids included in this study exhibited little structural resemblance to the existing COX inhibitors. However the flavonoids have shown interactions with COX-2 which are comparable to the synthetic drugs. Thus these compounds may serve as leads for the development of either selective or preferential COX-2 inhibitors containing a new nucleus. These results can also be used to explain the mechanism of action of the flavonoids.

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