RESEARCH ARTICLE

Molecular Docking studies of Novel Flavones as Cyclooxygenase- 2 (Cox 2) Inhibitors

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ABSTRACT

Cyclooxygenase (COX, prostaglandin endoperoxide synthase) enzymes catalyze the biological oxidation of arachidonic acid (AA) to prostaglandin H_2. These enzymes are biosynthesized in all tissues of the human body and elicit a variety of pharmacological effects. Some beneficial effects are the support of renal and platelet functions and gastrointestinal (GI) protection. Other non-beneficial effects are the pain, fever and symptoms associated with the inflammatory response. The enzyme COX-2 is an inducible form, expressed during inflammation. The aim of this study is to reveal the binding mode and hydrogen bond interactions of novel flavones with Cyclooxygenase- 2 enzyme. In the present study; we describe the molecular docking studies for a series of 25 novel flavones against COX-2 using Glide tool (Maestro ver. 9.10 of Schrodinger software). The docking results indicated that most of the compounds have shown hydrogen bond interactions with Tyr^{385},Tyr^{355},$Trp^{387}$and $Arg^{120}$and hydrophobic contacts with Val^{349},Leu^{352},Leu^{117},Tyr^{348} .
Hence, the above docking study results indicate that the binding affinity and hydrogen bond interactions of these molecules with respect to amino acid residues can be supportive evidence to carry out further studies in designing structure-based newer molecules with COX-2 inhibitory activity.

Keywords: Cyclooxygenase-2(COX-2), Flavones, Maestro, Docking, Glide.

INTRODUCTION

Cyclooxygenase (COX) is an enzyme that is responsible for the formation of prostanoids. The three main groups of prostanoids -- prostaglandins, prostacyclins, and thromboxanes - are each involved in the inflammatory response. [1] In the 1990s, researchers discovered that two different COX enzymes existed, now known as COX-1 and COX-2. Cyclooxygenase-1 (COX-1) is known to be present in most tissues. In the gastrointestinal tract, COX-1 maintains the normal lining of the stomach. The enzyme is also involved in kidney and platelet function. Cyclooxygenase-2 (COX-2) is present at sites of inflammation. [2] While both COX-1 and COX-2 convert arachidonic acid to prostaglandin, resulting in pain and inflammation; their other functions make inhibition of COX-1 undesirable while inhibition of COX-2 is considered desirable.

Nonsteroidal anti-inflammatory drugs (NSAIDs), commonly prescribed to treat arthritis, work by inhibiting prostaglandins. [3] Traditional NSAIDs (Ibuprofen, Naproxen), however, can cause gastrointestinal problems including ulcers. Traditional NSAIDs are considered nonselective because they inhibit both COX-1 and COX-2. The inhibition of COX-2 by traditional NSAIDs accounts for the anti-inflammatory effect of the drugs while the inhibition of COX-1 can lead to NSAIDs toxicity and associated side effects (ulcers, prolonged bleeding time, kidney problems). [3]

Flavones are the compounds belong to a widely distributed group of natural products called flavonoids with a variety of pharmacological activities including antioxidant, anticancer and anti-inflammatory activities. The anti-inflammatory activity is reported due to inhibition of lipooxygenase by the flavones. [4-5] Flavones are also known to suppress COX-2 transcription. [6] Literature reports that the anti-inflammatory and the anticancer activities of the flavones may be related to each other in view of the overexpression of COX-2 in some types of cancers. [7] In view of these literature findings, we decided to dock a series of flavones into the active site of COX-2 and study the binding pattern. Luteolin is a compound showing COX-2 inhibitory activity, [8] consisting of flavone moiety and SC-558 is selective

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COX-2 inhibitor used as reference ligands in this study. [9]

The aim of the present study is to conduct the molecular docking studies on a series of novel flavones as COX-2 inhibitors. The interest of the molecular docking studies is to know the binding mode of the ligands on COX-2 and the structural requirements important for COX-2 inhibition. Molecular docking studies were carried out to a data set of 25 molecules using GLIDE 9.1.

MATERIALS AND METHODS

The Docking studies were carried out by using GLIDE (Maestro, version 9.10, Schrödinger, LLC) 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 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 force field using standard protocol and parameters as included in GLIDE. The ligand structures were built, manipulated and adjusted for chemical correctness using Maestro 9.1 (Maestro, v9.1, Schrödinger LLC) graphical user interface. The ligands were geometry minimized using the OPLS_2005 force field and to a gradient RMSD below 0.01 kJ/Å.

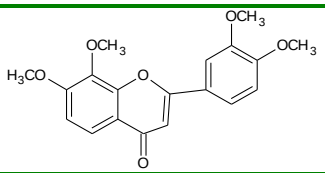
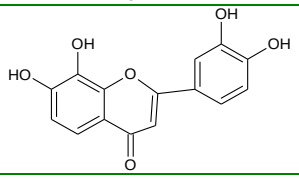
Receptor Grid Generation

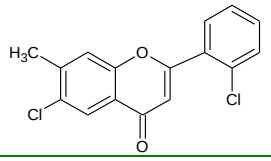
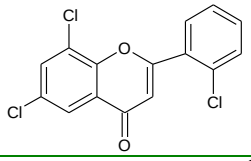
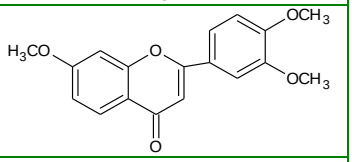
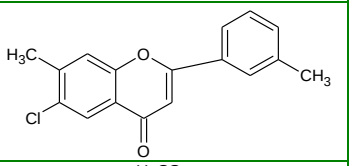
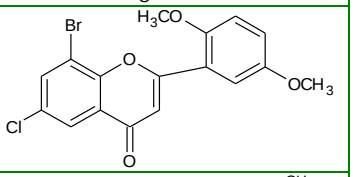
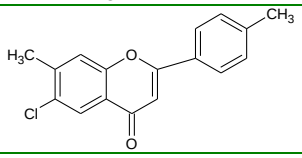
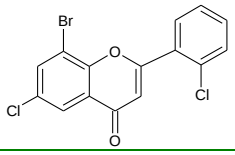
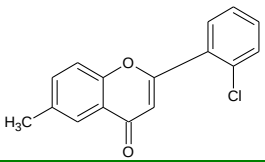
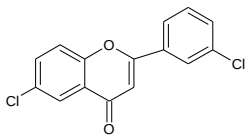
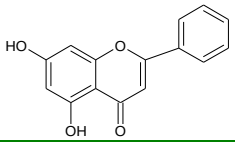
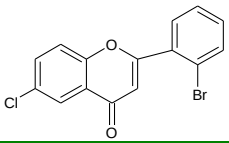
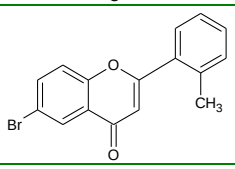
A grid was generated using the information on the crystal structure and docking information on the synthetic COX-1 and COX-2 inhibitors previously reported in the literature. Thus the active site comprised of His^{90} Arg^{120}, Tyr^{355}, Tyr^{385}, Arg^{513}, Val^{523} and Ser^{530}. [10]

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 0.52, thereby validating the docking protocol. The ligands selected for the present study consist of two types, viz. Synthetic flavones and reference ligands. The structures of 25 synthetic flavones and reference ligands have been shown in Table I. Table II shows G-scores and H bonds of synthetic flavones and reference ligands. Table III shows amino acids of COX-2 enzyme making good VDW contacts with flavones, SC-558, Luteolin and the number of bad and ugly vdw contacts. Fig. I-II indicates amino acid profile of the docked images of SC-558 and Luteolin. Fig. III-VII indicates amino acid profile of the docked images of synthetic flavones.

Table 1: It shows structures of synthetic flavones and reference ligands

S. No.	Compound Name and code	Structure
1	2-(3,4-Dimethoxyphenyl) - 7, 8-dimethoxy-4-chromenone (fl1)	
2	2-(3,4-Dihydroxyphenyl)- 7, 8-dihydroxy-4-chromenone (fl2)	

3	2-(6-Chlorophenyl)- 6-chloro-7-methyl -4-chromenone (fl3)	
4	2-(6-Chlorophenyl)- 6,8-dichloro-4-chromenone (fl4)	
5	2-(4,5- Dimethoxyphenyl) - 7-dimethoxy-4-chromenone (fl5)	
6	2-(5-Methylphenyl)- 6-chloro,7-methyl-4-chromenone (fl6)	
7	2-(2,5-Dimethoxyphenyl)-6-chloro-8-bromo-4-chromenone (fl7)	
8	2-(4-Methylphenyl)- 6-chloro-7-methyl-4-chromenone (fl8)	
9	2-(6-Chlorophenyl)- 6-chloro-8-bromo-4-chromenone (fl9)	
10	2-(6-Chlorophenyl)- 6-methyl-4-chromenone (fl10)	
11	2-(5-Chlorophenyl)-6-chloro-4-chromenone (fl11)	
12	2-(phenyl)- 5,7-dihydroxy-4-chromenone (fl12)	
13	2-(6-Bromophenyl)- 6-chloro-4-chromenone (fl13)	
14	2-(6-Methylphenyl)- 6-bromo-4-chromenone (fl14)	

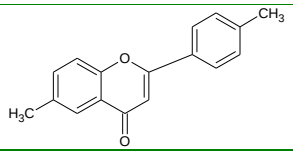
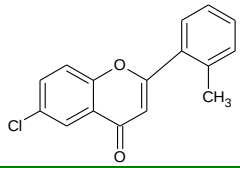
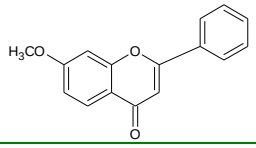
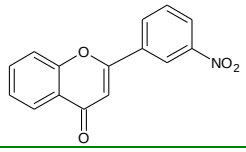
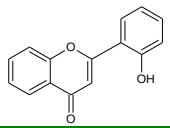
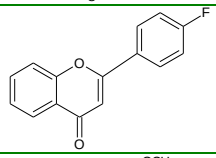
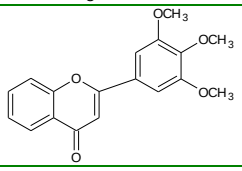
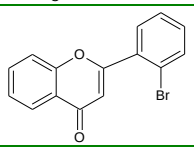
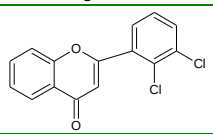
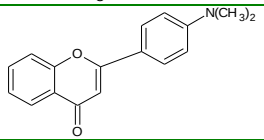
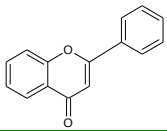
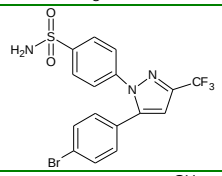
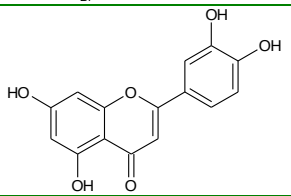
15	2-(4-Methylphenyl)- 6-methyl-4-chromenone (fl15)	
16	2-(6-Methylphenyl)- 6-chloro-4-chromenone (fl16)	
17	2-(phenyl)- 7-methoxy-4-chromenone (fl17)	
18	2-(5-Nitrophenyl)-4-chromenone (fl18)	
19	2-(6-Hydroxyphenyl)-4-chromenone (fl19)	
20	2-(4-Fluorophenyl)-4-chromenone (fl20)	
21	2-(3,4,5-Trimethoxyphenyl)-4-chromenone (fl21)	
22	2-(6-Bromophenyl)-4-chromenone (fl22)	
23	2-(5,6-Dichlorophenyl)-4-chromenone (fl23)	
24	2-(4-Dimethylaminophenyl)-4-chromenone (fl24)	
25	2-(phenyl)-4-chromenone (fl25)	
26	SC-558	
27	Luteolin	

Table 2: It shows Glide score (g-score) and number of H-bonds of synthetic flavones and reference ligands

Sr. No.	Compound code	G-score	H-bonds
1	fl1	-7.6970	0
2	fl2	-8.8411	1
3	fl3	-8.5300	1
4	fl4	-8.3466	1
5	fl5	-7.3342	0
6	fl6	-8.2979	1
7	fl7	-8.2196	1
8	fl8	-8.2134	1
9	fl9	-8.6262	1
10	fl10	-8.1400	1
11	fl11	-8.1117	1
12	fl12	-8.8209	1
13	fl13	-8.4507	2
14	fl14	-7.9426	0
15	fl15	-7.9417	1
16	fl16	-7.9068	1
17	fl17	-7.8983	1
18	fl18	-7.8960	0
19	fl19	-8.6483	1
20	fl20	-7.8370	0
21	fl21	-7.8368	1
22	fl22	-7.7090	2
23	fl23	-7.6648	1
24	fl24	-7.6604	1
25	fl25	-7.6222	1
26	SC-558	-10.107	3
27	Luteolin	-8.3212	1

Table 3: It shows amino acids of COX-2 enzyme making good VDW contacts with flavones, SC-558, Luteolin and the number of bad and ugly vdw contacts.

Compound code	Amino acids showing good vdw contacts with ligands	No. of Bad vdw contacts	No. of Ugly vdw contacts
fl1	Ser353,Leu531,Arg120,Val349,Leu352,Arg513, Leu531	3	0
fl2	Arg120,Leu531,Tyr355,Tyr385,Leu352,Ser353	0	0
fl3	Arg120,Val116,Ile345,Tyr355,Ser353,Met522, Leu384,Phe381	2	0
fl4	Arg120,Val116,Ser353,Leu352,Tyr385,Tyr348, Tyr355	2	0
fl5	Arg120,Tyr355,Leu352,Ser530,Ala527,Val349	2	0
fl6	Ser353,Arg120,Val349,Leu352,Leu117,Tyr355	1	0
fl7	Arg120,Tyr355,Tyr385,Leu352,Ser353,Arg513, Trp387	0	0
fl8	Arg120,Tyr355,Ser353,Leu352,Phe518,Met113, Leu117,Val349,Val523,Tyr38	3	0
fl9	Arg120,Val116,Ser353,Leu359,Tyr385,Leu352, Tyr348,Tyr355	1	0
fl10	Arg120,Tyr355,Leu352,Val116,Leu384,Phe381, Leu93	5	0
fl11	Trp387,Tyr385,Leu352,Gln192,Arg513,Tyr355, Val523,Ala527	7	0
fl12	Trp387,Leu384,Tyr385,Val349,Leu359,Arg120	1	0
fl13	Arg120,Val349,Tyr348Met522,Leu352,Phe381, Val116,Leu359	3	0
fl14	Arg120,Val349,Ser353,Leu384,Leu352,Leu359,Tyr355, Phe381,Val116	6	0
fl15	Arg120,Leu352,Ala527,Val349,Val116,Leu93	4	0
fl16	Ser353,Leu359,Arg120,Val116,Leu352,Leu384,	2	1
fl17	Arg120,Tyr355,Tyr385,Ser353Val349,Ala527	6	0
fl18	Arg120,Ser353,Leu352,Phe381,Gln192,Ala516,Arg513	3	0
fl19	Arg120,Val116,Leu359,Tyr385,Met113,Tyr355, Ile345,Leu531,	1	0
fl20	Arg120,Leu352,Ser353,Val116,Leu359,Phe381,Tyr385	4	0
fl21	Arg120,Tyr385,Leu352,Val116,Leu117,Met113	3	0
fl22	Arg120,Ile517,Ser353,Ala516,Ala527,Val349	7	0
fl23	Arg120,Ser353,Arg513,Ala516,Ala527,Val349,Tyr348	2	0
fl24	Arg120,Leu117,Val116,Leu352	1	0
fl25	Val349,Ala527,Phe381,Gly526,Leu384,Leu352, Arg120,Val116	2	0
SC-558	Tyr355,Arg120,Leu531,Val349,Tyr385	6	1
Luteolin	Tyr385,Ser530,Leu384,Leu359	4	0

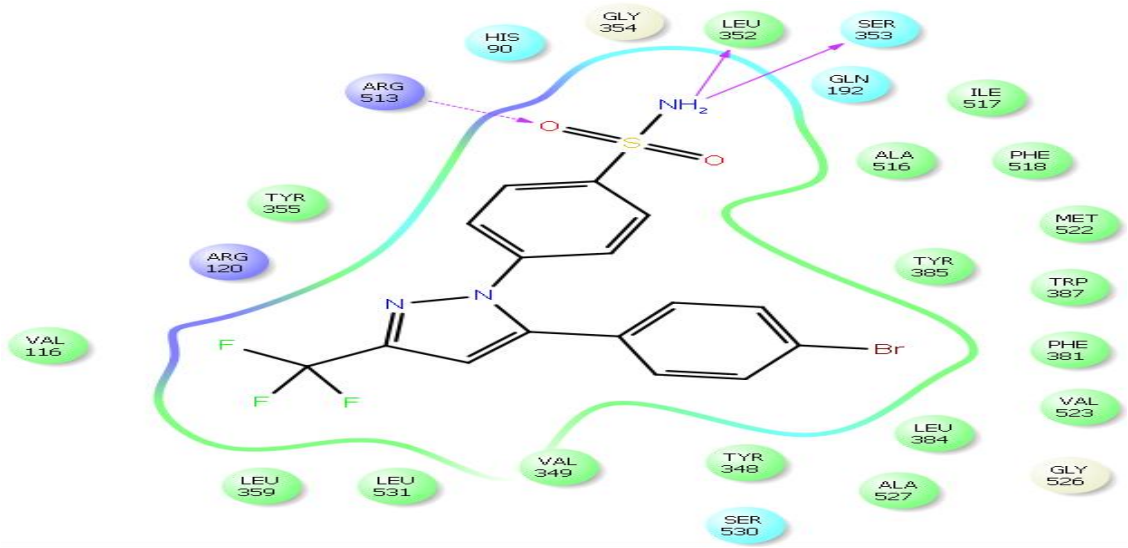


Fig.1: It shows SC-558 docked into the pocket of COX-2 enzyme

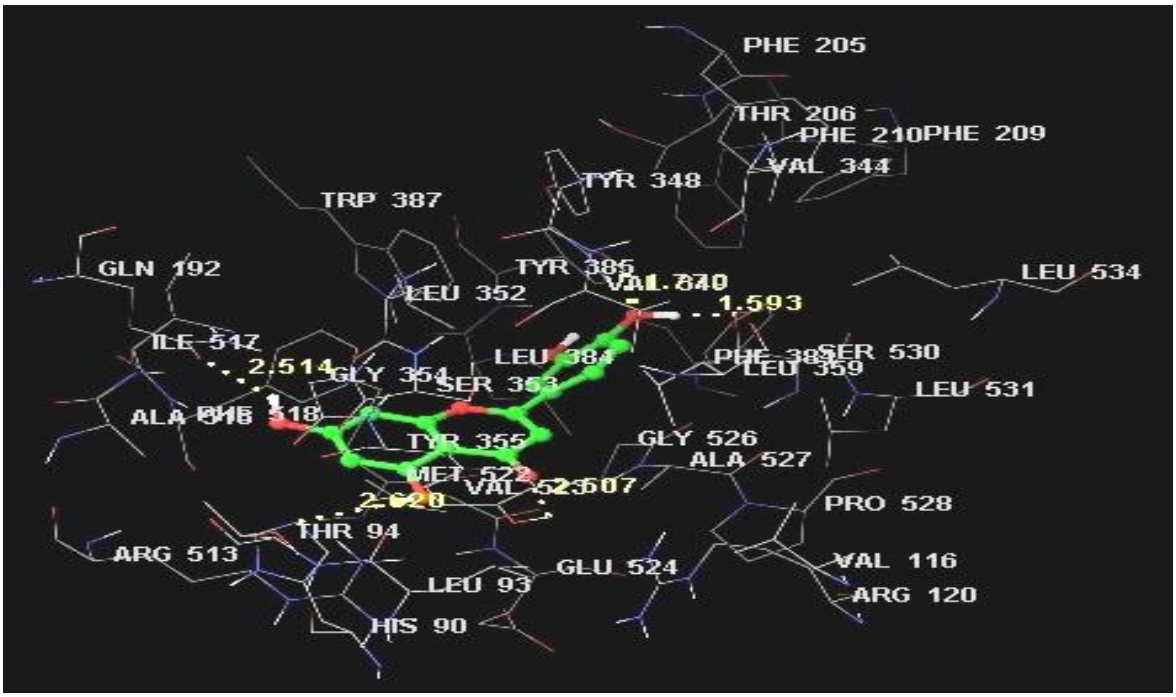


Fig. 2: It shows Luteolin docked into the pocket of COX-2 enzyme

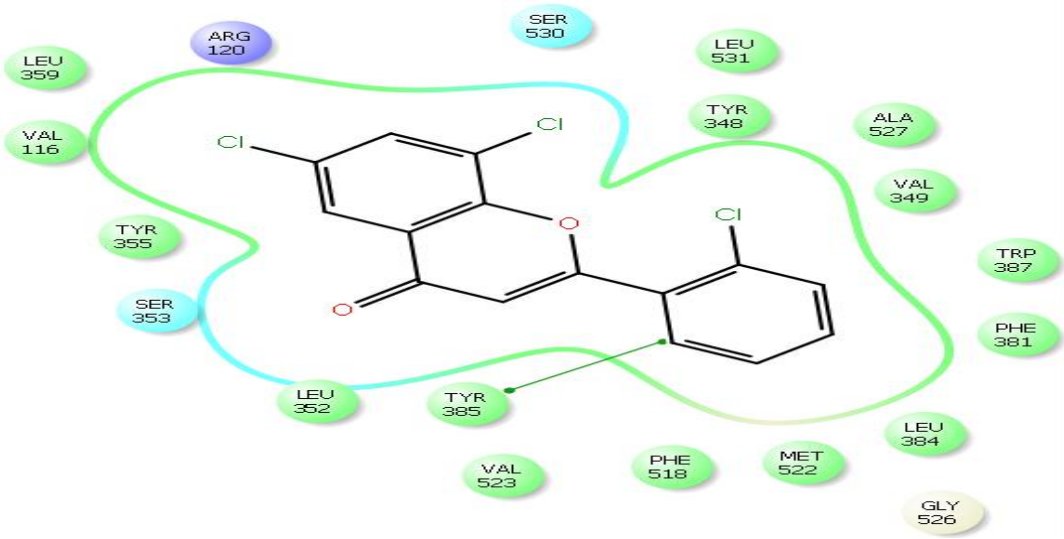


Fig.3: It shows compound fl4 docked into the pocket of COX-2 enzyme

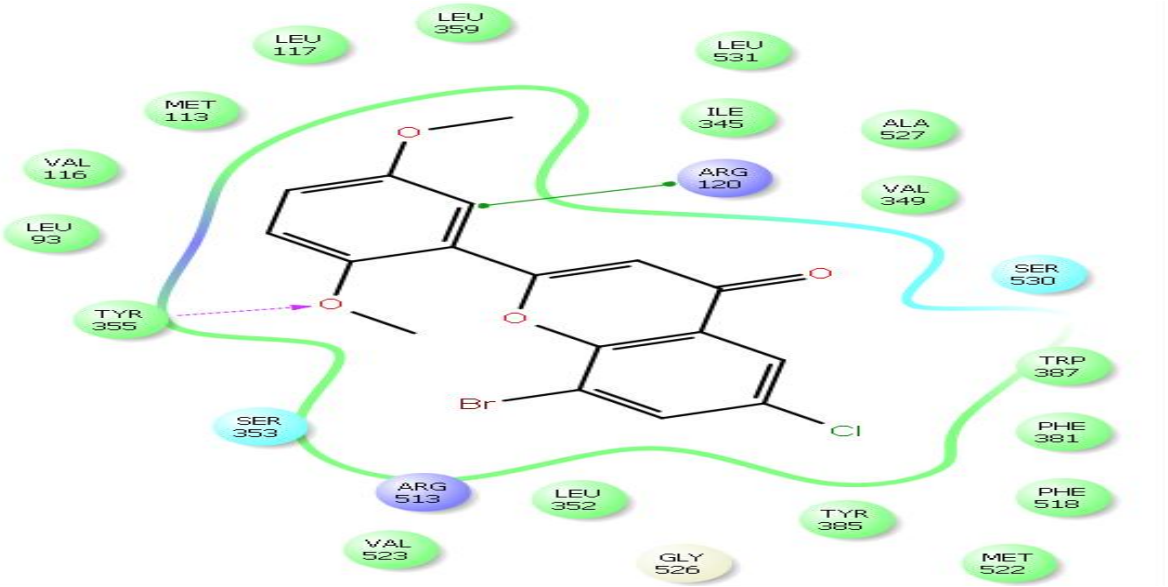


Fig 4: It shows compound fl7 docked into the active site of COX-2 enzyme

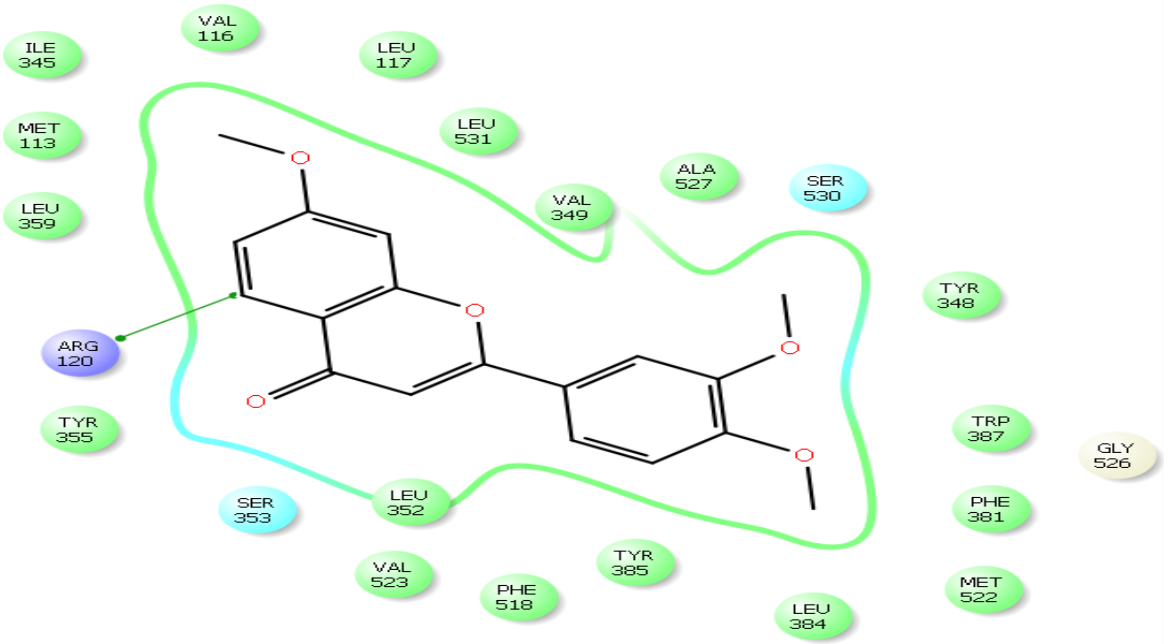


Fig. 5: It shows compound fl11 docked into the pocket of COX-2 enzyme

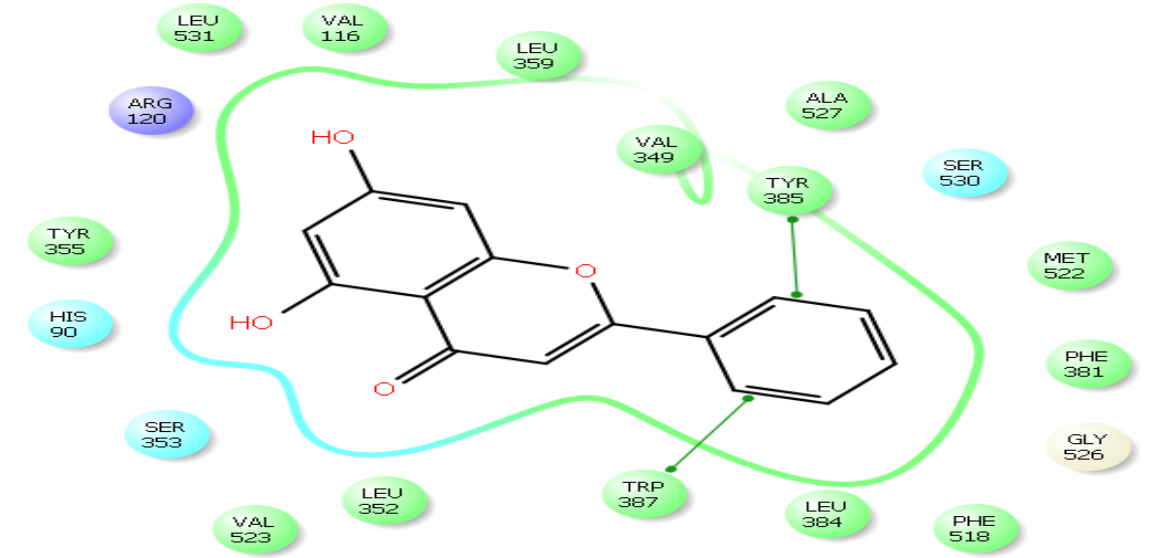


Fig. 6: It shows compound fl12 docked into the pocket of COX-2 enzyme

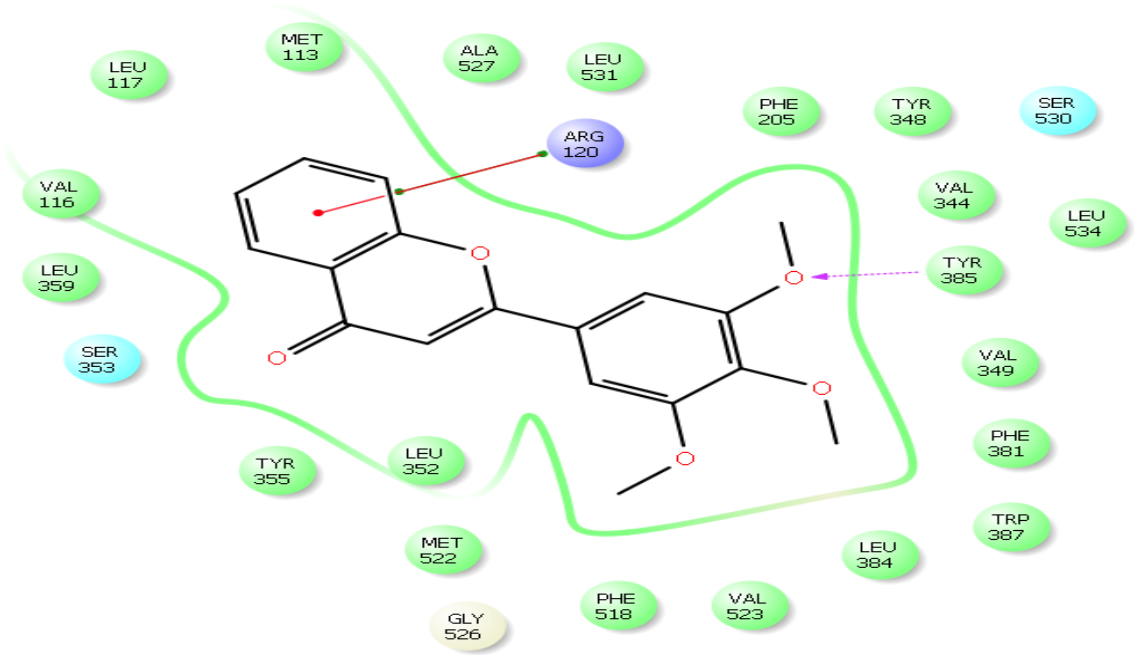


Fig.7: It shows compound fl21 docked into the pocket of COX-2 enzyme

RESULTS AND DISCUSSION

The active site of COX-2 is divided into three important regions, [11] 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}. 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 phenyl sulphonamide group occupied the side pocket and showed binding with Leu^{352}, Ser^{353} 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. [12]

One of the keys to developing COX-2 selective drugs is the larger active site of COX-2, which makes it possible to make molecules able to fit the COX-2. [13] The larger active site of COX-2 is partly due to a polar hydrophilic side-pocket that forms by Val^{523}, Arg^{513}, and Val^{434}. Val^{523} is less bulky which increases the volume of the active site. Val^{434} allows the side-chain of Phe^{518} to move back and make some extra space. This side-pocket allows for interactions with Arg^{513}. The side-chain of Leu^{384}, at the top of the receptor channel is oriented away from the active site and makes more space in the apex of the binding site in COX-2. [13, 14] Within the hydrophilic side-pocket of COX-2, 'A' ring of the flavone group interacts with Arg^{120} and forms hydrogen bonds. The synthetic flavones also interact with Tyr^{385},$Tyr^{355}$and Trp^{387}. The substituted phenyl group at the top of the channel interacts with the side-chains of amino acid residues through hydrophobic and electrostatic interactions. Tyr385 makes for some sterical restrictions of this side of the binding site so a small substituent of the phenyl group makes for better binding. Degrees of freedom are also important for the binding. The central ring of the flavones decides the orientation of the aromatic rings and, therefore, the binding to COX-2 enzyme even though it often has no electrostatic interactions with any of the amino acid residues.

CONCLUSION

In this study, molecular docking studies were carried out in a series of flavones using Glide software to describe the binding mechanism of ligands to the

target COX-2 enzyme. These docking results precisely indicate that novel synthetic flavones have good hydrogen bond interactions with Tyr^{385}, Tyr^{355}, Trp^{387} and $Arg^{120}$and hydrophobic contacts with Val^{349}, Leu^{352}, Leu^{117}, Tyr^{348} residues present at the catalytic site of the COX-2 enzyme. The generated molecular docking model has given scope for the development of novel chemical entities with potent COX-2 inhibitory activity.

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