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# DOCKING STUDIES OF CYCLOOXYGENASE 2 INHIBITORY ACTIVITY OF FLAVONOIDS

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## ABSTRACT

Docking of small molecules within the binding site of the macromolecules and estimation of the binding affinity of those compounds is a vital part of structure based drug design. The current study illustrates the binding of flavonoids against the cyclooxygenase enzyme using *in-silico* docking studies. For this purpose, apigenin, galangin, genistein, hesperitin, kaempferol, luteolin and quercetin were selected. In-silico docking studies were carried out using Molgro Virtual Docker (MVD) software.Docking result shows that all these flavonoids fits well in the active site of COX2 with Luteolin and Genisten with the highest docking score -102.99 Kcal/mol and -100.96 Kcal/mol, respectively, thus, could be potent inhibitor of COX2.

**Key words:** Cyclooxygenase2 (COX2), Molgro Virtual Docker (MVD), Flavonoids, docking studies

## INTRODUCTION

Drug design, an important tool in the field of medicinal chemistry, is used to find highly active compounds against a biological target with minimum side effects (Cavasotto and Abagyan, 2004). Now a days, the use of computers is an increasingly important component in the drug discovery process where binding affinity of small molecules to the known target proteins can be predicted (Schoichet, 2004; Koppen, 2009). Docking of small molecules in the receptor binding site and estimation of binding affinity of the complex is a vital part of structure based drug design (Seeliger and Groot, 2010).

Wide range of software are available for molecular docking simulations like, Gold (Jones *et al*., 1997), Dock (Ewing *et al.,* 2001), FlexX (Rarey *et al.,* 1996), Glide (Halgren *et al.,* 2004), Slide (Schnecke *et al*., 2000) , AutoDock (Morris *et al.,* 1998), Surflex (Jain, 2003) and MVD (Thomsen and Christensen, 2006). In the studies reported here, Molegro Virtual Docker (MVD) was used, because of its higher docking accuracy as compared to other available docking programs (Thomsen and Christensen, 2006)

Docking methods use an energy-based scoring function to identify energetically the most favorable conformation of a ligand when bound to the target macromolecule. Lower energy scores indicates more favored protein-ligand complexes. Thus, the task of molecular docking is to find the ligand binding mode with lowest potential energy. The process of docking involves identifying the target binding site and scoring each possible ligand pose within that site and the highest scoring pose is taken as the predicted binding mode for that compound.

Cyclooxygenase (COX) is a key enzyme in the conversion of arachidonic acid to prostaglandins. Two isoforms of COX have been identified and are designated as COX-1 and COX-2. COX-1 is expressed constitutively in various types of cells and tissues, including gastric mucosa (Williams and DuBois, 1996).

In contrast, COX-2 belongs to a class of genes referred to as immediate early or early growth response genes which are expressed rapidly after stimulation by growth factors, cytokines, and tumor promoters (Nathans *et al.* 1988; Herschman, 1991), therefore, COX-2 expression is increased at the sites of inflammation and in inflammatory cells (Masferrer *et al.,* 1994). Thus, COX2 enzyme plays a role in inflammation and tumor formation. (Subbaramaiah *et al*., 1997)

There are evidences that COX-2 is involved in tumorigenesis. For example, COX-2 is over-expressed in various solid tumors, (Sano, *et al*., 1995; Wolff, *et al*., 1998; Tucker *et al*., 1999; Mohammed *et al.,* 1999; Shirahama, 2000) and COX-2 inhibitors suppress tumor formation in experimental animals (Oshima *et al*., 1996; Okajima *et al*., 1998) and humans. (Giardiello *et al.,* 1993)

Several recent studies have reported a 40–50% decrease in the relative risk of colorectal cancer in persons who uses aspirin or other nonsteroidal antiinflammatory drugs (NSAIDs) continuously (Giovannucci *et al.,* 1994, 1995; Marnett,1992, 1995; Thun *et al.,* 1991, 1993), which suggest that these drugs serve as effective cancer chemopreventive agents. However, prolonged use of NSAIDs results in untoward gastrointestinal side effects that are due to inhibition of gastric prostaglandin production, which play a crucial role in maintaining gastric mucosal integrity. One common target of action for this class of drugs is the enzyme cyclooxygenase (COX).

There are now considerable evidences, from several different experimental systems, that COX-2 may play a role in the genesis of colorectal cancer (Reddy *et al.,* 1996; Prescott *et al.,* 1996; Oshima *et al.,* 1996; Tsujii *et al.,*

1995). Thus, inhibition of this enzyme specifically could be a possible strategy for the treatment of this disease. Therefore, during this study, flavonoids have been screened by performing Molecular docking using the Molegro Virtual Docker (MVD) software in order to search potential inhibitors of the enzyme COX2.

Flavonoids, a group of natural substances with variable phenolic structures, are found in various foods and beverages of plant origin (Middleton, 1998; Herrmann, 1976) and have many biological and pharmacological activities ; antibacterial, antiviral, antioxidant, and inhibition of several enzymes have been demonstrated (Vanden *et al.,* 1993; Bors *et al.,* 1990; Formica and Regelson, 1995). Furthermore, epidemiologic studies also suggest a protective role of dietary flavonoids against coronary heart disease (de Groot and Rauen, 1998)

The stereochemistry of binding of the flavonoids on cyclooxygenase has not yet been characterized. In the present study, the structural models of the ligands in the cyclooxygenase binding sites has been carried out, which may facilitate further development of more potent anti inflammatory agents.The main goal of this work is to obtain high efficiency, low toxicity colorectal cancer treatment drugs from flavonoid compounds by docking studies with the aim to inhibit COX-2 .

## MATERIAL AND METHODS

In this study, docking of selected flavonoids against COX2 has been performed using Molgro Virtual Docker (MVD) software. MVD uses a differential evolution algorithm and the energy function is the sum of inter-molecular interaction energy between protein and ligand with the intra-molecular interaction energy of the ligand. The docking energy scoring function used by MVD is derived from the PLP scoring functions originally proposed by Gehlhaar *et al.,*. (1995, 1998) and modified later by Yang and Chen (2004) with new hydrogen bonding and electrostatic terms included. During this study 10 solutions obtained from the 10 independent docking runs and are then re-ranked, in order to further increase the docking accuracy, by using a more complex scoring function. In MVD, along with the docking scoring function terms, a Lennard Jones 12-6 potential (Morris *et al.,* 1998) and $sp^2$-$sp^2$ torsion terms were also used.

The flavonoid like Apigenin, Galangin, Genistein, Hesperitin, Leuteolin, Kaempferol and Quercetin were selected for this study. The selected ligand structures were built using ChemDraw software (Figure 1) and optimized using “Prepare Ligands” in the MVD for docking studies.

Several crystal structures of COX2 have been solved by X-ray diffraction complexed with different ligands. The crystal structure selected for this study is 3PGH complexed with flurbiprofen (Figure 2). This crystal structure of COX2 (PDB code: 3PGH) downloaded to MVD workspace from Protein Databank .

For accurate docking it is important that the imported structures have been prepared properly, that is, the atom connectivity and bond orders are correct and partial atomic charges are assigned. PDB files often have poor or missing assignment of explicit hydrogens, and the PDB file format cannot accommodate bond order information. All necessary valency checks and H atom addition were thus performed using the utilities provided in MVD.

The pockets or cavities were identified in the crystal structure of COX2 by MVD using its cavity detection algorithm. The cavities within a 30 x 30 x 30 $Å^3$ cube centered at the experimentally known ligand position were used. The cavities that are identified by the cavity detection algorithm are then used by the guided differential evolution search algorithm to focus the search, to that specific area during the docking simulation.

The number of runs specifies the number of times that the docking simulation is repeated for each ligand chosen to be docked, 10 dockings runs have been performed here which come up with 10 solutions (poses). The docking poses were then ranked according to their docking scores

For each ligand docking, the best orientation for the ligand-protein complex were analyzed and hydrogen bonds were identified and labelled. The ligand energy was inspected and analyzed using MVD score, a linear combination of E-inter (steric, van der waals, H bondingand electrostatic interactions) and E-intra (torsion, sp2-sp2, hydrogen bonding, van der Waals and electrostatic interactions).

## RESULTS AND DISCUSSION

Cyclooxygenase (COX) exist in two isoforms designated as COX1 and COX2 and catalyses the conversion of arachidonic acid to prostaglandins. COX2 inhibition by the non-steroidal anti-inflammatory drugs (NSAIDs) provide the anti-inflammatory effects, however, concerns have found that COX2 inhibition by NSAIDs could have cardiovascular and renal adverse effects. Here, in this study *in-silico* investigation have been done in search of alternative potent COX2 inhibitors with reduced side effects. For this purpose COX2 was docked with the selected seven flavonoids (Figure 1). In each docking run, the best poses were selected on the basis of their MVD score.

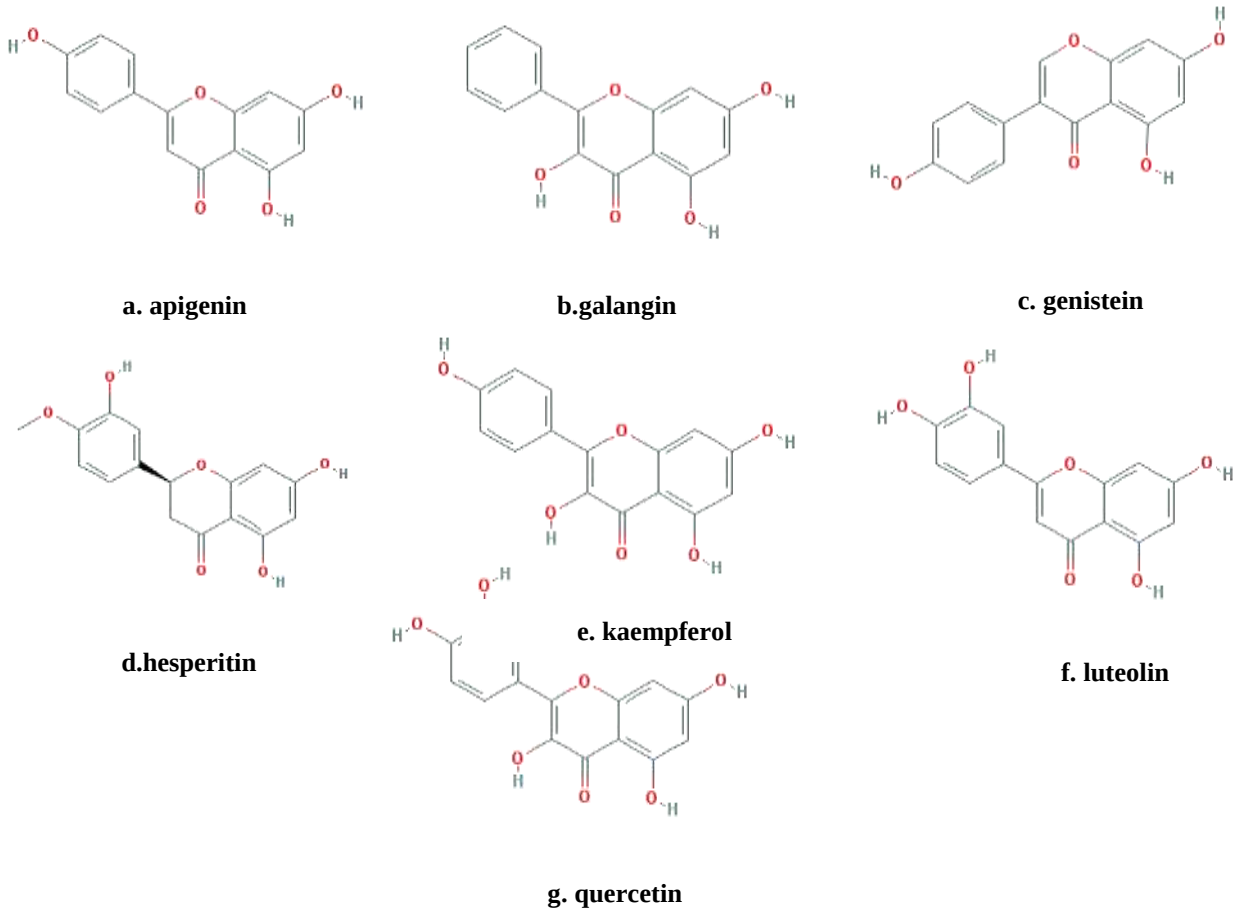



Fig. 1. Structure of flavonoids selected for docking against COX2.

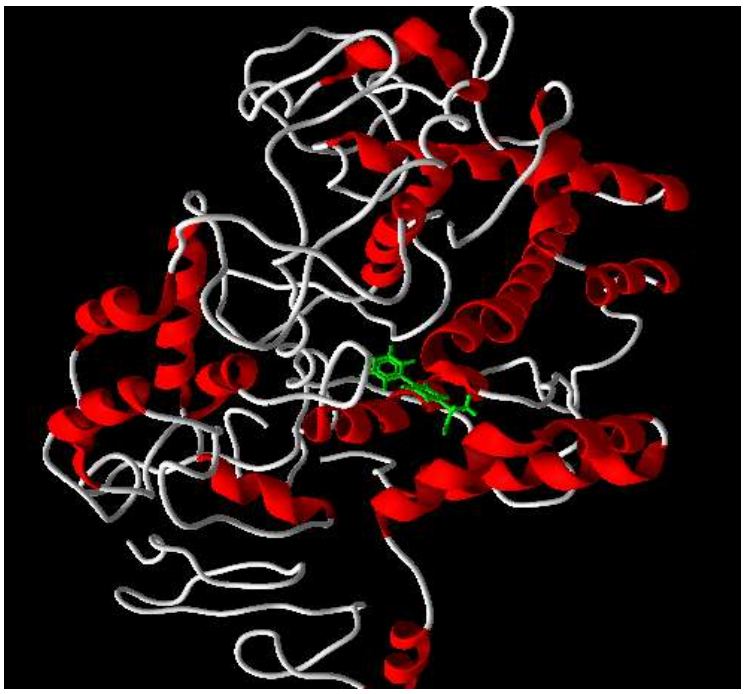
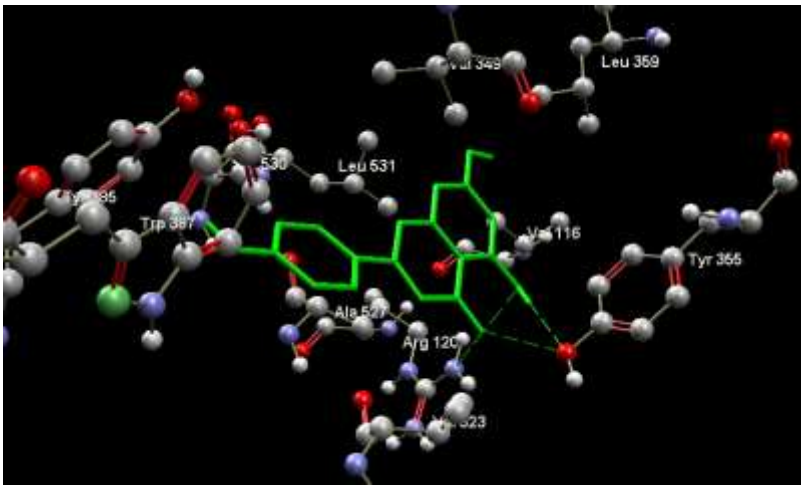
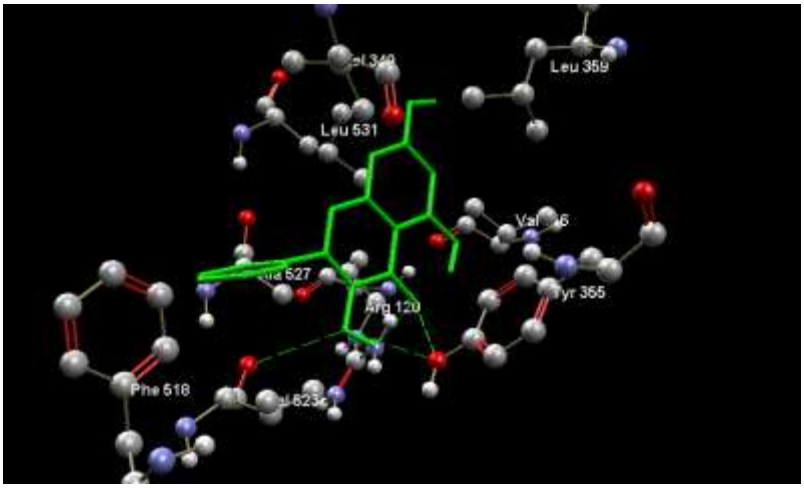


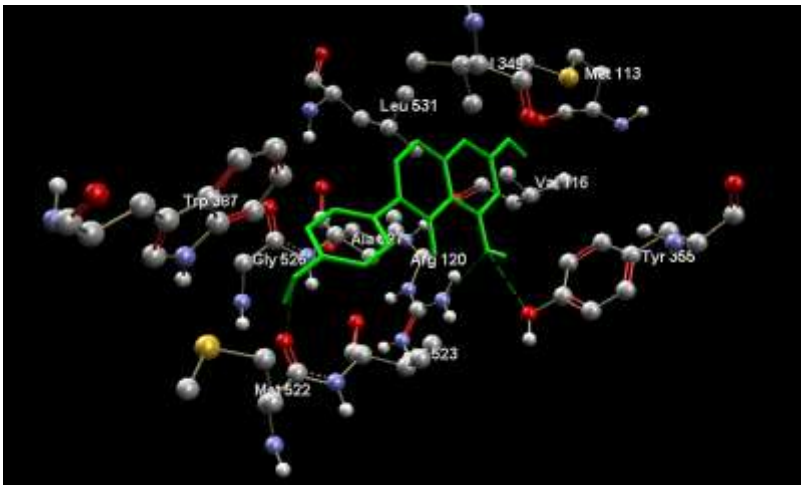
Fig. 2. Secondary structure of COX2 (PDB ID: 3PGH) complexed with flurbiprofen (green color).




**a. apigenin**

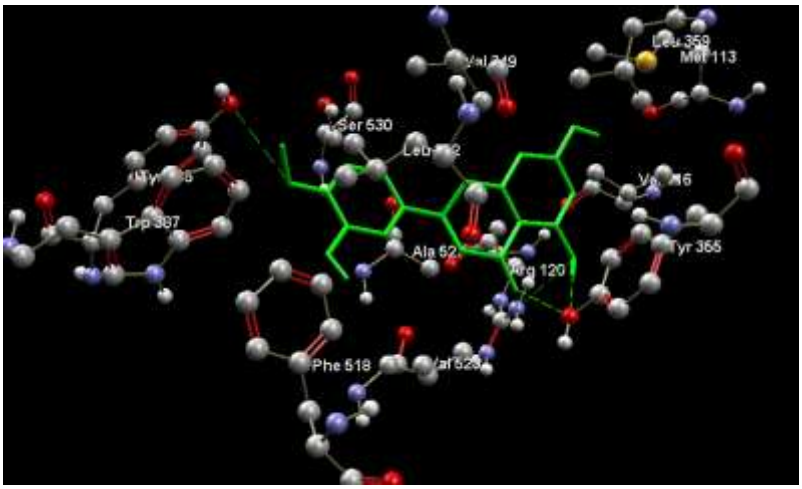



**b. galandin**

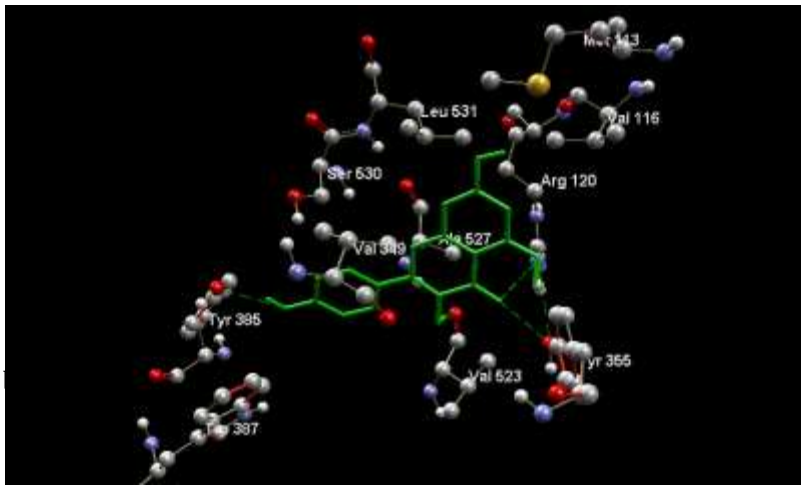



**c. genestin**

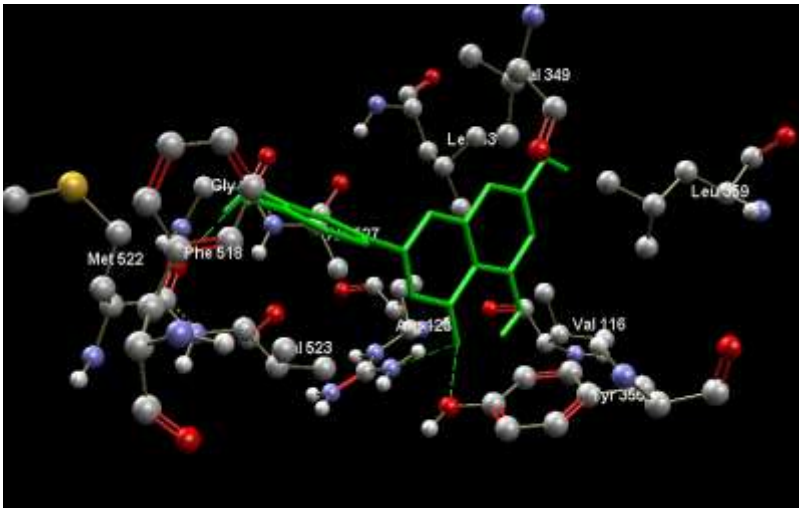
Fig.3.




**d. hesperitin**

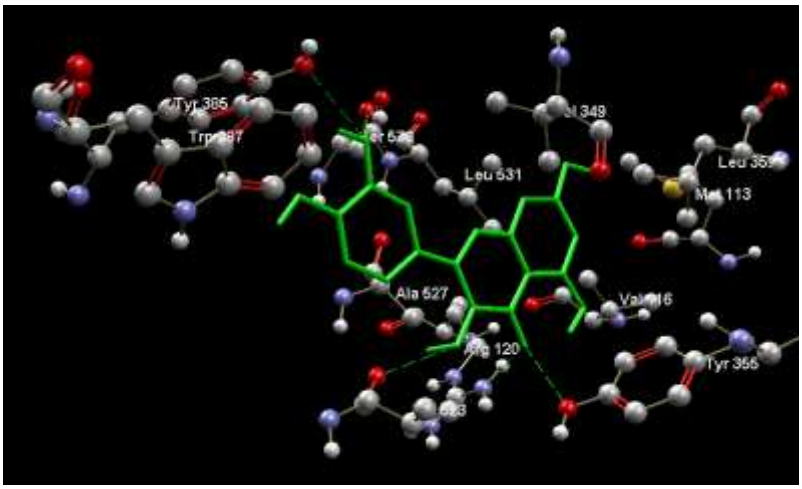



**e. kaempferol**




**f. leutolen**

Fig.3




**d. quercetin**

Fig. 3. The best scored docking solution of seven flavonoids (a to g) with the crystal structure of COX2. (Amino acids in the active site are presented in ball and stick with element color and the docked ligand and hydrogen bonds are presented in green).

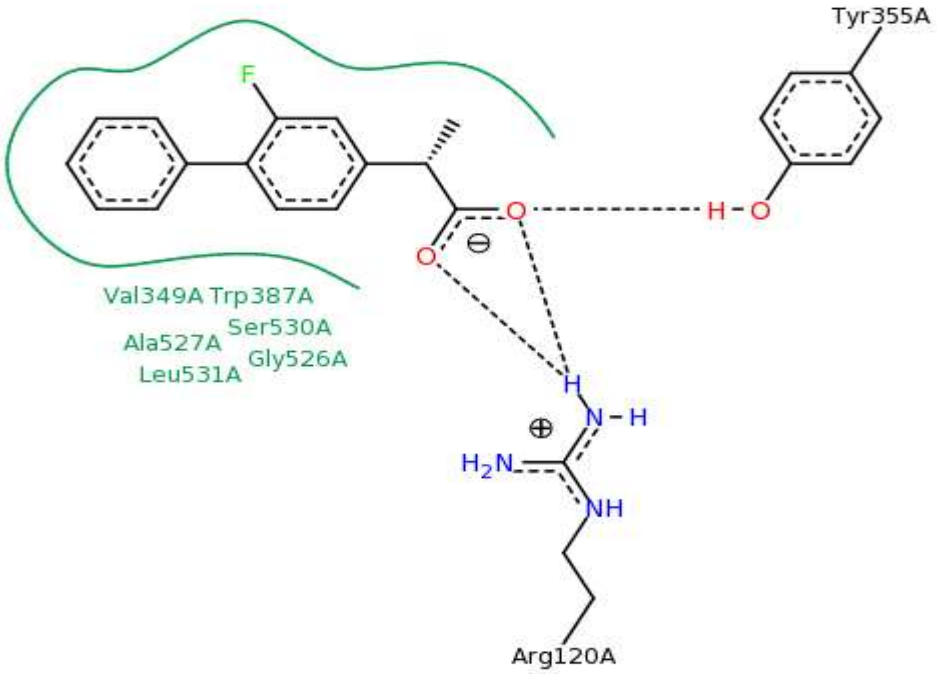



Fig. 4. Binding of flurbiprofen in the active site of COX2 (PDB ID:3 PGH).

The best docking poses obtained on the basis of MVD score for COX2 with each of the seven flavonoids are presented in Figure 4 and their MVD score, re-rank score and the hydrogen bond energies are presented in Table 1.

Analysis of the crystal structure of COX2 complex with flurbiprofen (Kurumbail *et al.*, 1996) reveals that the active site of COX2 is made of amino acid residues Val349, Trp387, Ala527, Leu531, Ser530 and Gly526 and the ligand (flurbiprofen) forms hydrogen bonds with the amino acids Arg120 and Tyr355 (Figure 4). These interactions are also found in the other crystal structures of COX2 bound to different ligands and are thought to be important for the binding of the ligand to the active site of COX2 and thus for their activity.

Table 1. MolDock score, rerank socre and the hydrogen bond enegy of the docked compounds.

| S.No | Ligands | MolDock Score | Rerank Score | Hbond |
|---|---|---|---|---|
| 1. | Apigenin | -98.99 | -86.695 | -1.828 |
| 2. | Galangin | -96.01 | -87.02 | -3.58 |
| 3. | Genistein | -100.96 | -88.29 | -3.127 |
| 4. | Hesperitin | -97.14 | -76.74 | -4.17 |
| 5. | Kaempferol | -98.06 | -87.06 | -3.62 |
| 6. | Luteolin | 102.987 | -89.18 | -3.69 |
| 7. | Quercetin | -93.58 | -80.52 | -6.93 |

All of the selected flavonoids when docked with COX2 they orientated in the same position in the active site as the bound ligand in the crystal structure and also forms hydrogen bonds with Arg120 and Tyr355 along with few other amino acids within the active site of this protein (Figure 3). Comparison of the docking score of these flavonoids shows that Luteolin and Genisten have the highest docking score (-102.99 Kcal/mol, and -100.96 Kcal/mol, respectively) and are thus predicted to be more potent as compared to other docked flavonoids

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