In Silico Approach: Docking Study of Compounds in Ardisia Plant as COX-2 Inhibitor and Its Comparison with Existing Therapeutic Drugs

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Abstract. Inflammation, a defense mechanism, happens during infections and injuries, involving complex immune responses. Medication using non-steroidal anti-inflammatory (NSAID) as Cyclooxygenase-2 (COX-2) inhibitors is clinically effective. It, however, has cardiovascular side effects. Thus, screening of COX-2 inhibitor from the nature, applying in silico study that is an effective and economical technique to reduce time-consuming in drug discovery process, is needed to be carried out to find drug candidates for anti-inflammatory with lower toxicity to cardiovascular. The virtual screening of ninety-two (92) compounds from Ardisia plants, which are *Ardisia humilis* Vahl and *Ardisia elliptica*, and current drugs for inhibiting COX-2 enzyme had been carried out using Molegro Virtual Docker v 7.0.0 (MVD), applying human COX-2 enzyme as target (PDB ID: 5IKQ). It, next, was visualized by performing PyMol software version 2.5.1. Lastly, a web-based prediction tools towards human ether-a-go-go related gene (hERG1) was used to study the cardiovascular toxicity, evaluating four best scores from Ardisia compounds and existing drugs. The results showed that the top four score compounds had been obtained and passed the Lipinski rule, including Isorhamnetin 3-sulfate, Isorhamnetin, Cornudentanone, and Rapanone, scoring of re-rank 115.27 kcal/mol, -105.87 kcal/mol, -105.10 kcal/mol and -104.38 kcal/mol, respectively. In addition, these compounds had shown comparative result with existing drugs such as Celecoxib, Rofecoxib, Meloxicam, Etodolac, and Meclofenamic acid. In addition, referring to the toxicity prediction, Isoharmnetin 3-sulfate and Rapanone showed a low-risk cardiovascular toxicity compared to existing drugs (Celecoxib and Etodolac). In silico study of compounds in Ardisia Plant, therefore, might assist further research to obtain potential candidates as anti-inflammatory drug with inferior side effect on cardiovascular.

Keywords: COX-2 · *Ardisia* humili Vahl · *Ardisia elliptica* · In Silico

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1 Introduction

Inflammation is a reaction of complexed immune responses to combat alien invasion, such as microbiology infection, stress, and self-attacking disease [1]. Inflammation, a reaction to protect the body, will cause four classical symptoms, namely fever, redness, pain, and swelling. These symptoms are caused by the defense mechanism activation, secreting biochemical markers such as vasoactive amine, peptide, and eicosanoids. Prostaglandin, an example of eicosanoids that shows a potent mediator involved in inflammation, is synthesized by hydrolyzing arachidonic acid (AA) with Cyclooxygenase (COX). COX, an endogenous enzyme, is divided into 2 isoforms, namely COX-1, and COX-2, which are responsible for converting AA to Prostaglandin G2 (PGG2) [2]. Inhibiting COX can be one of the solutions to reduce inflammation, which had been demonstrated by many drugs such as Aspirin, indomethacin and Non-Steroid Anti-Inflammatory Drugs (NSAIDs) [3, 4]. These drugs are the most frequent drug to be used for reducing inflammation through inhibiting prostaglandin production via COX. Existing anti-inflammatory drugs (COX-1 inhibitor), however, can stimulate adverse effects such as gastroduodenal bleeding, ulceration, and nephrotoxicity, if they repeatedly consumed [5]. In addition, COX-2 inhibitor drugs also lead to cardiovascular effect such as myocardial infarction due to having low anti-thrombotic activity [5, 6]. Thus, to fill in the gap, having effective compounds as anti-inflammation and tolerable side effects from natural products are required to be developed, selecting an in-silico approach as a way to maximize the outputs.

Herbal plants have been showing a promising target for COX-2 inhibitor [7–9]. *Ardisia*, one of herbal plants from Indonesia, is a plant that could grow until 6 m with its base diameter around 15 cm, dark brownish trunk, strong taproot with many branches, and rough and elastic texture leaves, which turns from light red to dark green when grow from young to old leaves [10] might have the potency to act as anti-inflammatory agent. *Ardisia* plants (*Ardisia humilis* Vahl and *Ardisia Elliptica*) are constituted more than 90 compounds from its leaf, fruit, and stem [11, 12]. Literatures highlighted that it could be applied for anti-viral [11], anti-bacterial [13, 14], anti-proliferative [15, 16], and anti-platelet [17]. Since inflammation is found in majority of infections and active compounds could act as anti-viral and anti-bacterial activity, a study using in silico approach to investigate the effect of *Ardisia* compounds on inflammation is required to be performed in regard to find potential compounds from *Ardisia*, which may also have minimum potency of side effect on cardiovascular compared to NSAIDs.

This study, therefore, was conducted to select and screen potential compounds from *Ardisia*, which have anti-inflammation activity, applying in-silico method. In addition, predicted binding sites of *Ardisia*'s compounds were also identified and compared to anti-inflammatory drugs. Lastly, the best scores of compounds from *Ardisia* against COX-2 enzyme and current drugs for anti-inflammation were furtherly studied to examine their cardiovascular toxicity effect.

2 Materials and Methods

2.1 Protein Preparation

Human COX-2 enzyme was used as target protein which was acquired from protein data bank (https://www.rcsb.org/pdb) (PDB ID: 5IKQ) with resolution 2.41 Å. Meclofenamic is a standard anti-inflammatory drug for this protein with JMS code as reference ligand. PDB of COX-2 was imported into MVD (Molegro Virtual Docker version 7.0 software) for protein preparation. Protein preparation was done with removing water and cofactor. Moreover, charge calculation and additional hydrogen was performed by molegro algoritma.

2.2 Ligand Preparation

Structure ligand was obtained from pubchem (https://pubchem.ncbi.nlm.nih.gov/) in the form of SMILE then was imported into Marvin sketch 17.27 software and it, then, was converted into 3D structure in mole.2 form. Then, the energy was minimized using dreiding force field in the same software.

2.3 Molecular Docking Studies for COX-2 Inhibitor

Before docking simulation, the validation method was carried out by redocking the reference ligand to protein COX-2 (PDB = 5IKQ) with coordinates based on reference ligand, x = 21.60 y- = 51.88 z = 17.70 radius 15. MolDock score (GRID) function was used with process GRID resolution was set 0.30 Å. Default parameter of MVD which composed of the number of steps per run (maximum iteration) 1500 with maximum population 50 and number of runs 10 with MolDock SE algoritm. The energy threshold was 100 for the minimized final orientation. The simplex evaluation with 300 maximum steps of neighbor distance factor 1 was completed. The result of validation method showed the RMSD was less than 2. Thereafter, total of 97 ligands of *Ardisia humilis* Vahl and *Ardisia elliptica* were docked using validated coordinates. Lastly, interaction between ligands and active sites of COX-2 enzyme was investigated to identify the hydrogen and steric interaction.

2.4 Cardiovascular Toxicity Analysis

Analysis cardiovascular toxicity was carried out by inhibition hERG channel using online server of PreADMET (https://preadmet.bmdrc.kr/). Here, the four best scores from docking simulation and existing drugs were analysed to investigate the cardiovascular toxicity.

3 Results

Docking results can be seen in Table 1 and 2. Table 1 shows the score of MolDock and re-rank of existing drugs as anti-inflammatory. Virtual screening of compounds was based on re-rank score which is the calculation for ligand-protein affinity, involving E-inter and I-intra. E-inter is constructed from steric, Van der Waals, hydrogen bonding and electrostatic between the ligand and the protein. In addition, E-intra is comprised of ligand measured by pre-determined coefficients, including hydrogen bonding, Van de Waals, rotation, sp2-sp2 and electrostatic. In addition, re-rank score is also calculated based on MolDock score, which is originally from the Piecewise Linear Potential (PLP) [18, 19]. The lower the energy of re-rank means that ligands might spontaneously adhere to active enzyme and have superior stability of the complex. Celecoxib was the lowest re-rank score (-128.16 kcal/mol) of existing drugs, followed by rofecoxib, meloxicam, etolodac, and reference ligand JMS_602 (-111.67 kcal/mol, -102.03 kcal/mol, -89.68 kcal/mol, and -88.98 kcal/mol, respectively) (Table 1).

Table 2 represents MolDock, Re-rank score, H-bonding of 92 compounds from *Ardisia*. It can be seen that Isoscutellarein 4'-methyl ether 8-(2'-sulfatoglucoside) Petunidin 3-O-beta-D-galactopyranoside, Peonidin-3-glucoside, Myricetin-3-O-arabinoside, Quercetin-3-O-glucoside were the 5 best score when it docked to human COX-2 enzyme. However, when applying the Lipinskyrule, the compounds that gave best results were 1) Isorhamnetin 3-sulfate, 2) Isorhamnetin, 3) Cornudentanone, and 4) Rapanone. Web-based study on cardiovascular toxicity found that Isoharmentin 3 -sulfate, Rapanone and Cornudentanone had low risk toxicity. In addition, existing drugs such as Celecoxib and Etodolac was categorised as medium risk toward cardiovascular toxicity (Table 3).

Table 1. Docking result of current drugs celecoxib, rofecoxib, meloxicam, etolodac, and reference ligand (meclofenamic acid) against COX-2

No	Ligand	MolDock score, kcal/mol	Re-rank score, kcal/mol	H-bond energy, kcal/mol	Lipinskyrule
1	Celecoxib	-157.48	-128.16	-2.55	Yes
2	Rofecoxib	-135.64	-111.67	-2.59	Yes
3	Meloxicam	-117.50	-102.03	-1.04	Yes
4	Etodolac	-113.27	-89.68	0	Yes
5	JMS_602 (A)	-107.66	-88.98	-0.81	Yes

Table 2. MolDock score and re-rank score (kcal/mol) for compound in *Ardisia* Plant docked toward COX-2 enzyme crystal structure

No.	Ligand	Plant clasification *	MolDock score, kcal/mol	Re-rank score. Kcal/mol	H-bond energy, kcal/mol	Lipinsky rule
1	Isoscutellarein 4'-methyl ether 8-(2'-sulfatoglucoside)	AE	-152.26	-127.43	-8.47	No
2	Petunidin 3-O-beta-D-galactopyranoside	AH	-167.59	-125.21	-11.48	No
3	Peonidin-3-glucoside	AE	-160.63	-124.68	-9.59	No
4	Myricetin-3-O-arabinoside	AE	-152.14	-124.66	-11.61	No
5	Quercetin-3-O-glucoside	AE	-138.26	-122.43	-3.97	No
6	Epigallocatechin-3-gallate isomer	AE	-162.93	-120.75	-11.87	No
7	Oxycoccicyanin	AH	-151.47	-116.57	-5.23	No
8	Isorhamnetin 3-sulfate	AE	-146.02	-115.27	-9.27	Yes
9	Quercetin-3-O-arabinoside	AE	-144.45	-114.65	-8.74	No
10	Quercetin-3-O-rhamnoside	AE	-144.64	-114.34	-10.70	No
11	Gossypetin 8-glucoside-3-sulfate	AE	-123.71	-113.51	-8.94	No
12	5-Pentadecylresorcinol	AE	-139.93	-107.35	-1.33	No
13	Quercetin 3-O-sulfate	AE	-131.96	-106.89	-13.29	No
14	Isorhamnetin	AE	-121.37	-105.87	-3.65	Yes
15	Cornudentanone	AE	-146.90	-105.10	-3.04	Yes
16	Rapanone	AE	-140.11	-104.38	-4.68	Yes
17	7,2'-Dihydroxyflavone 7-glucoside	AE	-121.92	-104.28	-3.66	No
18	(-)-Epigallocatechin-3-gallate	AE	-155.50	-104.26	-11.03	No
19	Gingerol	AE	-128.44	-101.72	-4.01	Yes
20	Quercetin	AE	-116.94	-100.83	-9.74	No
21	Monogalloylglucose	AE	-115.49	-99.38	-9.17	No
22	1,5-Dibutyl methyl hydroxycitrate	AE	-124.75	-99.29	-2.69	Yes
23	Kaempferol	AE/AH	-113.59	-98.82	-8.77	Yes
24	Ardisinol II	AE	-126.50	-98.17	-3.65	No
25	Catechin	AE	-115.64	-97.99	-6.48	No
26	5,7-Dimethoxyflavone	AE	-117.36	-97.84	0.00	Yes
27	Triangularin	AE/AH	-108.12	-97.52	0.00	Yes
28	1-Naphthyl ester acetoxyacetic acid	AE	-112.32	-97.36	-2.53	0
29	Myricetin-3-O-rhamnoside	AE	-124.67	-97.36	-11.15	No
30	Apigenin 7-sulfate	AE	-116.51	-97.25	-7.59	Yes
31	Kaempferol-3-O-rhamnoside	AE	-130.89	-95.35	-4.33	No
32	Salicyl acyl glucuronide	AE	-99.48	-94.83	-4.15	No
33	Luteolin 7-sulfate	AE	-122.10	-94.36	-11.72	Yes

(*continued*)

Table 2. (*continued*)

No.	Ligand	Plant clasification *	MolDock score, kcal/mol	Re-rank score. Kcal/mol	H-bond energy, kcal/mol	Lipinsky rule
34	Embelin	AE	-116.66	-94.22	-4.22	Yes
35	Ardisiphenol B	AE	-136.33	-92.77	-2.63	No
36	Acerosin	AE	-113.58	-92.53	-5.09	Yes
37	6-Chlorocatechin	AE	-117.73	-91.39	-10.51	No
38	4-t-Butyl-2-(4-nitrophenyl) phenol	AE	-103.34	-89.86	-3.53	Yes
39	Butyl octadecenoate	AE	-122.65	-89.63	0.00	No
40	4-Cyanophenyl 2,6-difluorobenzoic acid	AE	-104.32	-88.39	-1.28	0
41	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	AE	-131.29	-87.93	-2.40	No
42	Rhamnocitrin 3-O-sulfate	AE	-116.59	-87.76	-1.73	Yes
43	Hexadecanoic acid	AE	-111.81	-87.74	0.00	No
44	Tridec-2-ynyl 2.6-difluorobenzoic acid	AE	-123.41	-86.26	-3.48	0
45	n-Tridecanoic acid methyl ester	AE	-109.12	-86.16	-1.67	Yes
46	Bilobol	AE	-129.22	-85.71	-1.34	No
47	Pentadecanal	AE	-106.29	-85.57	0.00	No
48	Chlorfenapyr	AE	-136.77	-85.19	-0.16	Yes
49	2- Nonylmalonic acid	AH	-101.49	-85.13	-0.83	0
50	Myricetin-3-O-glucoside	AE	-146.16	-84.98	-11.13	No
51	5-(Z-heptadec-4'enyl)resorcinol	AE	-132.68	-84.42	-5.42	0
52	Berberin	AE	-128.03	-79.81	-2.61	Yes
53	Ardisisanone A	AE	-124.74	-78.35	-0.66	No
54	Primulin	AH	-141.62	-76.78	-13.51	No
55	2-Nonylmalonic acid	AE	-92.50	-76.66	-0.18	0
56	Bergenin	AE	-93.46	-75.68	-9.89	No
57	3,5-Di-t-butylphenol	AE	-92.43	-75.67	-2.50	Yes
58	Alpha tocopherol	AE	-132.96	-72.87	-2.12	No
59	Quercetin-3-O-rutinoside (Rutin)	AE	-139.01	-70.68	-11.57	No
60	Citric acid	AE	-72.19	-69.62	-6.35	Yes
61	Syringic acid	AE	-76.69	-69.41	-1.55	Yes
62	2,4-bis(1,1-dimethylethyl)-phenol	AE	-77.50	-66.90	0.00	Yes
63	Longifolenaldehyde	AE	-90.84	-63.64	-0.16	Yes
64	5-Hydroxymeth 2-furancarboxaldehyde	AE	-68.91	-58.71	-5.00	0
65	Clindamycin	AE	-125.72	-50.85	-2.50	Yes
66	Myricetin-3-O-rutinoside	AE	-156.15	-48.38	-20.07	No

(*continued*)

Table 2. (*continued*)

No.	Ligand	Plant clasification *	MolDock score, kcal/mol	Re-rank score. Kcal/mol	H-bond energy, kcal/mol	Lipinsky rule
67	Oenin	AH	-125.71	-47.06	-6.30	No
68	Aspidin	AE/AH	-104.74	-36.63	0.90	Yes
69	(+)-Catechin 6-C-glucoside	AE	-108.22	-35.46	-9.20	No
70	Maesaquinone	AE	-114.96	-33.55	-0.76	No
71	Giberelin A4	AE	-94.99	-31.86	-3.20	Yes
72	Ardisiaquinone D	AE	-106.73	-20.92	-0.71	No
73	Stigmasta-7–22-dien-3-ol	AE/AH	-126.05	-10.95	-4.56	Yes
74	Cycloheterophyliin	AE	-126.90	-8.62	1.55	No
75	Myrtillin	AH	-121.25	7.46	-13.36	No
76	Quercetin 3-lathyroside	AE	-167.45	11.99	-16.10	No
77	Decamethyl-cyclopentasiloxane	AE	-89.63	27.48	0.00	Yes
78	Squalene	AE	-124.40	31.96	0.00	No
79	Ardisiaquinone A	AE	-103.58	96.53	-2.60	No
80	Dodecamethylcyclohexasiloxane	AE	-90.47	103.76	-0.08	Yes
81	Friedelan-3-one	AE	-127.07	110.06	-0.16	No
82	Ardisenone	AE	-118.71	131.63	-1.04	No
83	Alpha-amyrin	AE/AH	-109.44	151.54	0.00	No
84	Alpha Amyrenol	AE/AH	-109.64	160.84	0.00	No
85	Formononetin 7-O-(2″-p hydroxybenzoylglucoside)	AE	-102.01	161.15	-6.30	No
86	Ardisiaquinone G	AE	-85.64	178.86	-5.26	No
87	Beta amyrin	AE	-111.70	236.88	0.00	No
88	Bauerenol	AE	-92.87	268.03	-5.96	Yes
89	Alpha amyrin acetate	AE	-97.01	384.08	-0.96	No
90	Thonningianin B	AE	-44.22	425.67	-3.57	No
91	Theasinensin A	AE	-32.01	983.05	-6.69	No
92	Ardisianoside D	AE	235.74	1512.23	-0.92	No

* AE = *Ardisia elliptica*
* AH = *Ardisia humilis Vahl*

Table 3. Cardiotoxicity prediction in hERG channel inhibition of current anti-inflammatory drugs and four-top docking result (COX-2 enzyme)

Ligand	Cardiotoxicity prediction
Celexocib	Medium risk
Rofexocib	Low risk
Meloxicam	Low risk
Etodolac	Medium risk
Isorhamnetin 3-sulfate	Low risk
Isorhamnetin	Medium risk
Cornudentanone	Low Risk
Rapanone	Low risk

4 Discussion

NSAIDs work as anti-inflammation, acting to inhibit prostaglandin synthesis from arachidonate acid, in which COX-1 and COX-2 are the contributed enzymes for the synthesis. However, NSAIDs that developed to selectively constrain the COX-2 have a side effect to cardiovascular. In this study, in silico method to study anti-inflammatory activity of *Ardisia* plants (*Ardisia humilis* Vahl and *Ardisia elliptica*) were conducted to obtain potential compounds with lower side effect to cardiovascular. When applying the Lipinskyrules, the result shows that Isorhamnetin 3-sulfate gave lower energy score which was similar compared to existing drugs, obtaining re-rank score -115.27 kcal/mol. Other best scores were, next, followed by Isorhamnetin, Cornudentanone, and Rapanone. Here, this study highlighted that Isorhamnetin 3-sulfate potentially acted as anti-inflammation agent with low cardiovascular toxicity.

It is postulated that these compounds that had lower re-rank score were bond with many active sites of COX-2 enzyme. Hydrogen bonds of Isorhamnetin 3-sulfate were with Gln 192, Phe 518, Ile 517, His 90, Ser 353, Tyr 355, Ser 530 of targeted enzyme (Table 4 and Fig. 1). Cornudentanone also had hydrogen bonds with amino acid of Leu 531, Ser 530, and Tyr 385 (Table 4 and Fig. 1). It is known that interaction in Ser 530 dan Tyr 385 of COX-2 enzyme can inhibit the catalytic activity [20]. In addition, hydrogen bond with Ser 530 dan Tyr 385 also was seen to interact with ligand reference and current inflammatory drugs such as Meloxicam dan Etodolac. Furthermore, Tyr 385 in the radical form is responsible to abstract proton from arachidonate acid during the conversion of PGG2 [21]. Those interactions possibly can hinder the activity to produce PGG2 due to inactivity of COX-2 enzyme.

Table 4. The interaction (hydrogen bond and steric bond) between ligand and amino acid of COX-2 enzyme

Ligand name	Hydrogen bond interaction	Steric bond interaction
JMS (A) Refference Ligand	Ser 530, Tyr 385	Ser 530, Val 349
Celecoxib	Phe 518, Tyr 355, Gln 192, Ser 353	Phe 518, Gln 192, Ile 517, Val 523, Leu 352, Ser 353
Meloxicam	Ser 530	Gly 526, Phe 381, Ser 530, Tyr 385, Val 349, Phe 518, Val 523, Leu 352, Gln 192, Arg 513, His 90,
Isorhamnetin 3-sulfate	Gln 192, Phe 518, Ile 517, His 90, Ser 353, Tyr 355, Ser 530	Ala 516, His Phe 390, Phe 518, Val 523, Phe 381, Leu 352, Val 349, Ser 353, Glu 524, Met 522
Isorhamnetin	His 90, Tyr 355	His 90, Val 523, Leu 352, Gly 526, Tyr 385, Leu 384
Cornudentanone	Leu 531, Ser 530, Tyr 385	Tyr 355, Val 523, Leu 531, Met 522, Tyr 385, Ser 530, Val 344, Val 349, Tyr 348, Trp 387, Leu 352, Leu 531
Rapanone	Leu 352, Arg 513, His 90, Tyr 355	Val 523, Phe 518, His 90, Leu 352, Ser 353, Met 522, Tyr 385, Tyr 348

Other compounds also adhere to other amino acid of COX-2 enzyme, Isorhamnetin, which was the second place after Isorhamnetin 3-sulfate, was bond with His 90 dan Tyr 355 (Table 4 and Fig. 1). The fourth rank was Rapanone which was connected to Leu 352, Arg 513, His 90, and Tyr 355 (Table 4 and Fig. 1). Study highlighted those amino acids of His 90 and Arg 513 are the hydrophilic side of COX-2 enzyme [22]. Thus, the interaction in these amino acids could hinder the enzymatic activity.

Literatures note that drugs to inhibit COX-2 enzyme have the potency to promote cardiovascular side effects [5, 6]. An approach to explore cardiotoxicity is through hERG potassium channel, which plays an eminent role in cardiac function, conducting electrical activity in the heart [23, 24]. If the inhibition hERG channel occurs, it can cause QT (a measured time of a cycle in cardiogram between wave Q and wave T) interval prolongation, which can increase cardiac arrhythmias or torsades de pointes (TDP) [25]. Thus, this study employed a cardiovascular toxicity prediction by performing an inhibition of hERG channel using web-based study (https://preadmet.bmdrc.kr/). The result showed that low risk toward hERG was obtained by Isorhamnetin 3-sulfate and Rapanone rather than Celecoxib and Etodolac, which had medium risk. This study, therefore, demonstrated that isorhamnetin 3-sulfate could perform as anti-inflammation compound and had lesser side toxicity effect to cardiovascular compared to commercial drugs for inflammation.

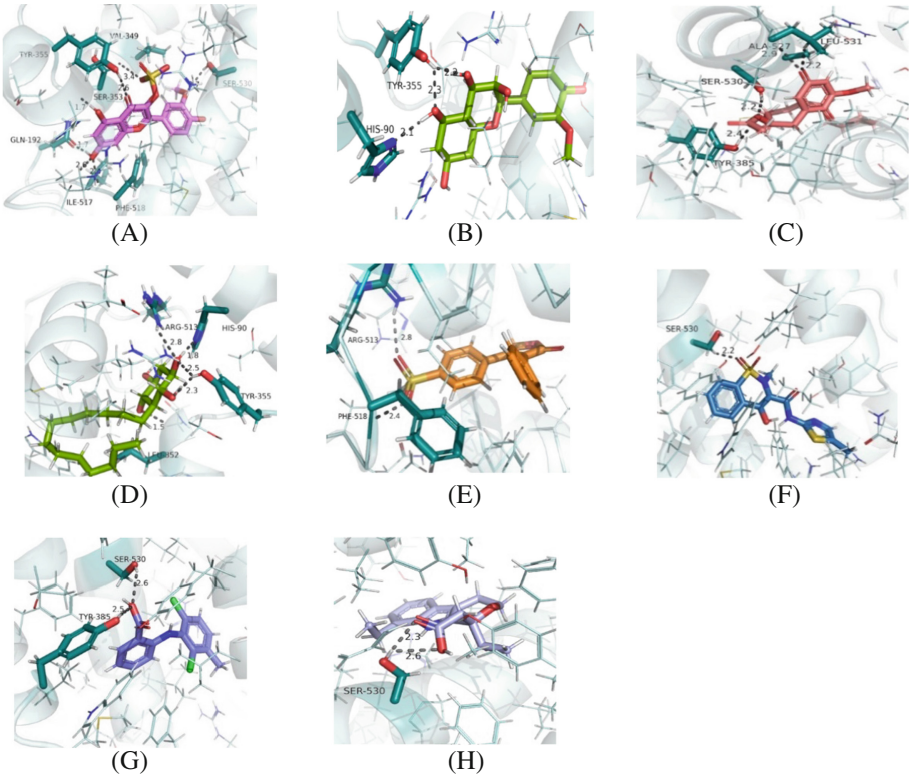

Fig. 1. The interaction visualization between ligands (A) Isorhamnetin 3-Sulfat, B) Isorhamnetin, C) Curnodentanone, D) Rapanone, E) Rofexocib, F) Meloxicam, G) Meclofenamic Acid, H) Etodolac) and amino acid of COX-2 Enzyme

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