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RESEARCH ARTICLE

In silico Investigation of Bioactive Compounds from *Ginkgo biloba* as Alternatives to Non-Steroidal Anti-Inflammatory Drugs

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A B S T R A C T

Non-steroidal Anti-inflammatory Drugs (NSAIDs) are common over-the-counter drugs that are used for numerous inflammation-associated ailments. Despite their widespread consumption, these synthetic drugs are not without side effects. Adversities caused by NSAIDs range from simple nausea and vomiting to fatal conditions, such as hypertension, gastrointestinal bleeding, and diminished renal function. Thus, there is a need to develop novel alternatives to these drugs that possess comparable efficacies. Phytocompounds are attractive alternatives for a plethora of medicines used for various disorders and diseases as they are readily available in nature and have negligible side effects. In an attempt to identify safe alternatives to NSAIDs, we tested six bioactive compounds from *Ginkgo biloba* (Ginkgolide A, Amentoflavone, Bilobetin, Ginkgetin, Quercetin, and Bilobalide) for their abilities to inhibit Cyclooxygenase-1, Cyclooxygenase-2 and 5-Lipoxygenase which are inflammation-causing enzymes. Molecular docking experiments using Autodock Vina resulted in binding energy values between -6.6 and -11.9 kcal/mol, comparable to that of control drugs, which indicated that the tested phytocompounds were able to bind strongly to the active sites of the three proteins. Analyses of receptor-ligand interactions using Discovery Studio Visualizer revealed that all the tested compounds formed numerous non-covalent interactions with the surrounding amino acid residues, which confirmed their binding stabilities. Finally, an evaluation of their drug-likeness based on Lipinski's rule of five showed that five of the tested *G. biloba* compounds possess the potential to be taken as oral drugs to replace conventional NSAIDs.

K E Y W O R D S

NSAIDs; Molecular Docking; Phytocompounds; Receptor-ligand Interactions; Drug-Likeness

H I G H L I G H T S

- Phytocompounds have the potential to replace NSAIDs since they induce negligible side effects.
- 6 phytocompounds from *Ginkgo biloba* are tested to inhibit enzymes causing inflammation.
- All of the phytocompounds can bind easily to the enzymes and form stable complexes.
- All of the phytocompounds except for amentoflavone is safe for oral consumption.

INTRODUCTION

Non-steroidal anti-inflammatory Drugs (NSAIDs) are a class of drugs commonly used for the treatment of conditions associated with inflammation. Examples of these include common over-the-counter

drugs, such as ibuprofen, celecoxib, benzydamine, naproxen, aspirin, diclofenac, etoricoxib, and indomethacin (NHS UK, 2019). NSAIDs act through competitive inhibition of inflammation-generating enzymes. Among these are the two forms of cyclooxygenase (COX), namely COX-1 and COX-2, which participate in the biosynthesis of prostaglandins (PGs), prostanoids and thromboxane (TX) via the arachidonic acid pathway (Gunaydin & Bilge, 2018) and 5-lipoxygenase (LOX), an enzyme involved in the conversion of arachidonic acid (AA) into leukotriene (LT) (Hu & Ma, 2018). thus, NSAIDs are capable of affecting the synthesis of various critical pyretic, analgesic and inflammation mediators; PGs, prostanoids, TXs and LT, along with other inflammatory mediators, such as interleukin-1β (IL-1β) and tumour necrosis factor (TNF) (Gunaydin & Bilge, 2018).

Despite the widespread use of NSAIDs, they impose unwanted side effects, such as cellular oxidative stress, leading to gastrointestinal bleeding and eventually gastritis, hyperkalemia, dyspnea, respiratory depression, diminished renal function, nausea, vomiting, esophageal irritation, abdominal pain as well as hypertension (Wishart et al., 2008). In addition, the use of NSAIDs can lead to preventable adverse drug reactions (PADRs), which are ADRs that are a consequence of medical errors. For example, PADRs may be a consequence of incorrect medication type, timing or dose, method of administration of medication or insufficient monitoring of medication (Davis & Robson, 2016; Wolfe et al., 2018) and account for 10% of hospital admissions, especially in the case of older individuals. Therefore, it is the need of the hour to identify safe alternatives that possess comparable efficacies to that of NSAIDs.

Phytochemicals (compounds of plant origin) have gained substantial interest recently due to their high efficacy in treating a spectrum of maladies with low or no adverse effects (Forni et al., 2019; Hossen et al., 2020; Zhang et al., 2015). *Ginkgo biloba*, commonly called maidenhair tree, is a woody plant native to the Chinese region that is rich in several bioactive compounds, including flavonoids, biflavonoids and terpenoids (Son et al., 2005; Tao et al., 2019). *G. biloba* has been employed for the treatment of a number of conditions such as dizziness, headache, depression, cough, anxiety, as well as problems related to memory and concentration (Chan et al., 2007; Shah et al., 2011; Tulsulkar & Shah, 2013). A study by Tulsulkar and Shah (2013) suggests that a *G. biloba* extract (EGb 761) exhibits neuroprotective effects in various models of ischemia. Additionally, EGb 761 is able to potentially reduce oxidative stress by reactive oxygen species and nitric oxide (Ilhan et al., 2018; Tian et al., 2013). A computational study performed by Mukhi and Jai (2020) showed that a phytochemical from *G. biloba*, Ginkgolide A, possesses the potential to be used as a treatment regimen for Huntington's disease.

Despite the wide usage of *G. biloba* as a treatment for various diseases, *G. biloba* can affect the serotonergic, adrenergic, and hypothalamic-pituitary-adrenal (H-P-A) axis system, which can negatively impact mood symptoms (Das et al., 2022). Additionally, a three-month *in vitro* study using *G. biloba* found that for both sexes of rats and mice, increased liver weight and hepatocytic hypertrophy rate were found to lead to decreased survivability if prolonged for two years. However, an *ex vivo* study using human endothelial artery cells conducted by Zhu et al. (2013) showed that *G. biloba* extract (GBE) and aspirin administered at therapeutic doses possess synergistic effects and are able to reduce oxidative stress through the activation of platelets by the regulation of ROS production and LOX-1 receptor. Moreover, GBE was found to be capable of inhibiting platelet aggregation in a dose-dependent manner. In relation to this, a review by Das et al. (2022) suggests that clinical trials conducted using specific doses of *G. biloba* produced a positive outcome. For example, patients with a generalized anxiety disorder (GAD) treated with 240 mg or 480 mg placebo of EGb 761 for four weeks have improved cognitive abilities along with reduced anxiety.

The anti-inflammatory properties of *G. biloba* have been demonstrated in several *in vivo* investigations. In a carrageenan model of acute inflammation, the therapeutic effects of EGb 761 on pain and inflammation were compared with those of diclofenac (Biddlestone et al., 2007) and piroxicam (Abdulrazak et al., 2018) as standard non-steroidal anti-inflammatory drugs. EGb-761 reduces acute inflammation in a dose-dependent manner and is comparable to diclofenac and piroxicam. A study conducted by Li et al. (2019)

showed that bilobetin obtained from *G. biloba effectively* inhibits COX-2 mRNA levels in a dose-dependent manner. Moreover, *in vitro* studies on other compounds obtained from *G. biloba*, such as amentoflavone and ginkgetin, showed an inhibitory effect on cyclooxygenase. In contrast, quercetin showed an inhibitory effect on both cyclooxygenase and lipoxygenase (Kim et al., 1998).

In an attempt to identify novel alternatives for NSAIDs, we examined the ability of six phytochemicals from *Ginkgo biloba* (Ginkgolide A, Amentoflavone, Bilobetin, Ginkgetin, Quercetin, and Bilobalide) for their abilities to inhibit inflammation-related enzymes such as LOX and COXs. This was performed by making use of computational tools, specifically molecular docking and the evaluation of their drug-likeness according to Lipinski's rule of five. Such *in silico* techniques have long been used for Computer-aided Drug Discovery as a first step in the drug-discovery pipeline. This paper thus proposes phytochemicals from *G. biloba* as alternatives to NSAIDs by investigating the natural anti-inflammatory medicine by inhibiting COX and LOX.

MATERIAL AND METHODS

Proteins and compounds used for the study

Molecular docking studies were performed using protein structures downloaded from the Protein Data Bank (https://www.rcsb.org/). The proteins selected were *Homo sapiens* cyclooxygenase-1 (COX-1), *Homo sapiens* cyclooxygenase-2 (COX-2), and *Homo sapiens* 5-Lipoxygenase (LOX) with PDB IDs 6Y3C, 5IKR, and 6N2W, respectively. The ligand structures were downloaded from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). The compounds selected were Ginkgolide A (Compound ID: 9909368), Amentoflavone (Compound ID: 5281600), Bilobetin (Compound ID: 5315459), Ginkgetin (Compound ID: 5271805), Quercetin (Compound ID: 5280343), and Bilobalide (Compound ID: 73581) (Figure 1), where Table 1 shows their pharmacological properties, which explain why these compounds were selected. The controls used were ibuprofen for COX-1, celecoxib for COX-2, and benzydamine for LOX (Wishart et al., 2008). Their structures were also obtained from the PubChem database; ibuprofen (Compound ID: 3672), celecoxib (Compound ID: 2662), and benzydamine (Compound ID: 12555).

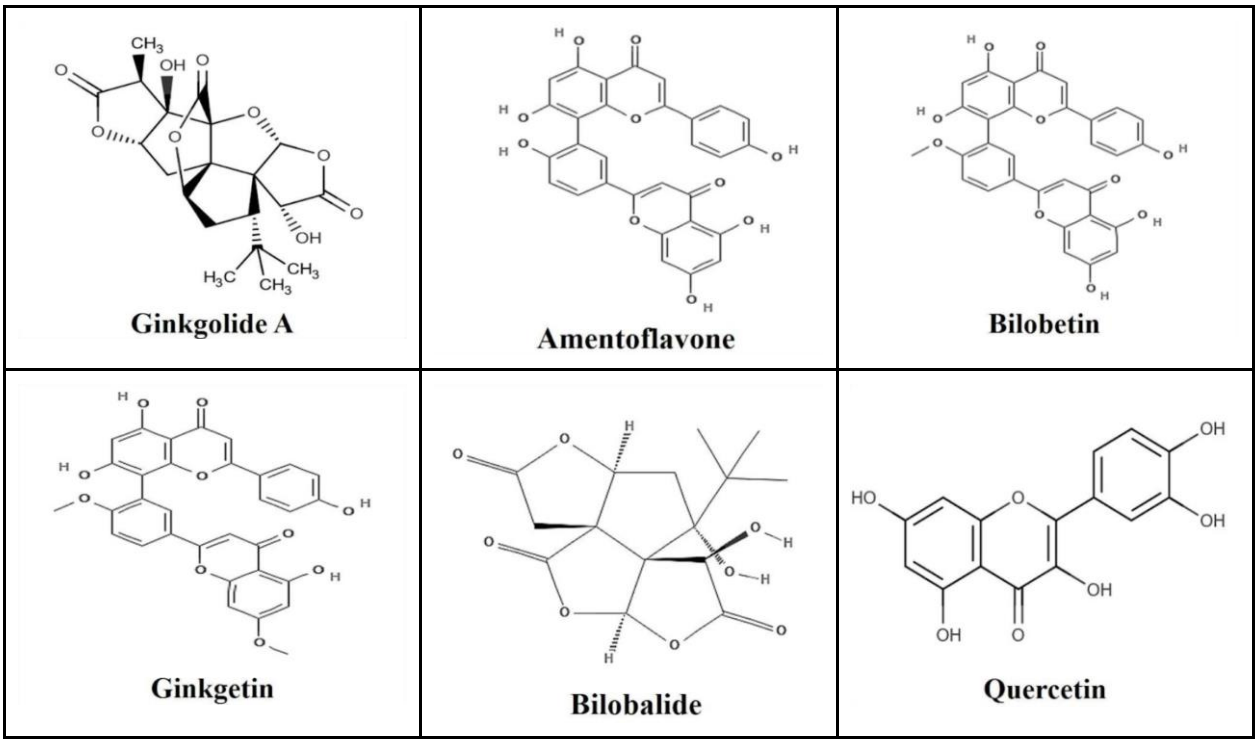

Figure 1. Structures of compounds selected from *G.biloba*

Table 1. Inclusion and exclusion criteria for considering studies

Compound	Chemical Species	Pharmacological Properties
Ginkgolide A	terpene lactone	Highly active PAF (Platelet Activating Factor) antagonist cafe molecule & potent therapy in inflammatory disorders
Amentoflavone	flavonoid	Biflavonoid, inhibit group II phospholipase A2 and cyclooxygenase
Bilobetin	flavonoid	Biflavonoid, increase PPARa (Peroxisome Proliferator-Activated Receptor Alpha) activity
Ginkgetin	flavonoid	A biflavonoid with inhibitory activities against COX-2
Bilobalide	sesquiterpene	Terpenoid which antagonizes the action of GABA (Gamma-Aminobutyric Acid), which modulates inflammation

Structure processing

Prior to docking, the downloaded protein structures were cleaned by removing all non-protein atoms (heteroatoms or HETATMs) using a text editor. The cleaned protein structures were then processed in AutoDock Tools 1.5.6 (Pacheco & Hpc, 2012), in which hydrogen atoms, as well as charges (Gasteiger and Kollman), were added to the proteins, after which the charges were spread uniformly throughout all the amino acid residues. Ligands structures were first energy minimized using Avogadro (Hanwell et al., 2012) to obtain their lowest energy states. This was done to avoid steric clashes or any other conformational abnormalities occurring in the ligand structures. Ligands for docking were then processed and generated using Autodock Tools 1.5.6.

Grid preparation and molecular docking

Information regarding the active site residues of each protein was obtained from LigPlot diagrams from the PDBsum database (http://www.ebi.ac.uk/thornton-srv/databases/cgi-bin/pdbsum/GetPage.pl?pdbcode=index.html). Grids encompassing all the active site residues were then created for each protein using Autodock Tools 1.5.6 to define the area for molecular docking. Table 2 summarizes the active site residues and the grid size (x, y, z) for all three enzymes. For all the docking experiments, energy range and exhaustiveness were set as 4 and 8, respectively. All the phytocompounds, as well as respective controls, were then docked with the three proteins using Autodock Vina (Allouche, 2012). Only the best binding poses and their corresponding docking scores were considered for further steps. Following docking, receptor-ligand interactions in all the docked complexes were visualized using Discovery Studio Visualizer 2021 (Client) (D Studio, 2008).

Table 2. Active site residues and grid sizes of COX-1, COX-2, and LOX for molecular docking

Enzyme	PDB Code	Active Site Residues	Grid Size (x,y,z coordinates in Å)	Center Grid Box (x,y,z coordinates)
COX-1	6Y3C	Chain A: Glu 140, Asn 144, Tyr 147, Met 216 and Phe 220 Chain B: -	42 x 34 x 40	x center : -30.895 y center : -68.601 z center : -09.382
COX-2	5IKR	Chain A: Glu 140, Asn 144, Tyr 147, Arg 216, Phe 220 Chain B: Leu 238 and Arg 242	40 x 68 x 38	x center : 29.940 y center : 31.947 z center : 54.499
LOX	6N2W	Chain A: - Chain B: Phe 359, Gln 363, Leu 368, His 372, Ile 406, Ala 410, Pro 569, Arg 596, Trp 599, His 600, Leu 607 and Ile 673	48 x 44 x 72	x center : 35.098 y center : 67.222 z center : 37.866

Grid box evaluation

The validation of docking experiment was done to validate the docking procedure to ensure the generation of validated data. Re-docking of native ligands of the proteins into the active sites by AutoDock Vina were utilized (Shivanika et al., 2020). The grid box used in this experiment covered up all parts of the protein; thus, blind docking was performed. The native ligand of each protein was first selected from RCSB PDB (https://www.rcsb.org/). The ligands were then extracted from the protein utilizing PyMol and were processed by AutoDock Tools to generate the PDBQT file format. Furthermore, the re-docking of the processed native ligands against each protein, namely COX-1, COX-2, and LOX, was done by AutoDock Vina, employing the same grid box used for the molecular docking of *G. biloba*'s active compounds. Results were then visualized using Discovery Studio Visualizer 2021.

Drug-likeness evaluation

Lipinski's rule of five was used to evaluate the ability of the six phytochemicals to potentially serve as oral drugs (Drug-Likeness). Molecular weight, number of hydrogen bond acceptors, number of hydrogen bond donors, and lipophilicity were calculated for each of the tested compounds using the SwissADME webserver (http://www.swissadme.ch/).

Toxicity evaluation

The toxicity of each bioactive compound selected from *G. biloba* was determined using the Pro Tox-II webserver (https://tox-new.charite.de/protox_II/). Utilizing the "Tox Prediction'' menu as per their respective Pubchem IDs. The toxicity information was then obtained from the web server. The toxicity categories, along with their LD50 values and explanations as per the Globally Harmonized System of Classification and Labeling of Chemicals (GHS) by the United Nations (2011), could be determined, as seen in Table 3.

Table 3. Categorization of Toxicity Evaluation

Toxicity Category	Lethal Dose (mg/kg)	Legend
Category I	LD50 ≤ 5	Fatal if swallowed
Category II	5 < LD50 ≤ 50	Fatal if swallowed
Category III	50 < LD50 ≤ 300	Toxic if swallowed
Category IV	300 < LD50 ≤ 2000	Harmful if swallowed
Category V	LD50 > 2000	May be harmful if swallowed

RESULTS AND DISCUSSION

Molecular docking

Molecular docking has been long regarded as a promising tool for drug design involving the analysis of the binding of a ligand with a target protein or receptor (Cheke, 2020). Through molecular docking, the interactions between two binding molecules can be virtually modelled (Pantsar & Poso, 2018). Protein-ligand binding can only occur when the change in free Gibbs energy is negative, which spontaneously favors the product formation (the protein-ligand complex) (Du et al., 2016). Thus, high binding affinity is characterized by low binding energy. Through the use of molecular docking in our study, it was found that all the tested phytocompounds, when docked with the proteins, resulted in large and negative binding energy values, indicating that they bound strongly to their targets. Furthermore, all the binding energy values obtained were comparable to those of the control drugs.

As shown in Table 4, amentoflavone had the highest binding affinity for all three enzymes among all the tested ligands and control drugs, while bilobalide showed the lowest binding affinities, which were nevertheless comparable to the controls. In the case of COX-1, all the tested phytochemicals had larger binding energy values than the control. This result indicated that any of the compounds from *G. biloba* would serve as effective alternatives to ibuprofen. In the case of COX-2, except bilobalide, all the tested compounds showed larger binding affinities than the control. However, the binding energy of bilobalide was comparable (-8.3 kcal/mol) to that of the control (-8.8 kcal/mol). Finally, in the case of LOX, all the tested compounds showed equal or higher affinities compared to the control. The binding energy values obtained suggested that the tested phytocompounds have the potential to bind to the inflammation-related enzymes and inhibit their activities, thus mimicking the abilities of conventional NSAIDs.

Table 4. Binding Energy Values of Controls and *G. biloba* compounds docked to COX-1, COX-2 and LOX (kcal/mol)

Protein	Control	Active Compounds of *G. biloba*					
		Ginkgolide A	Amentoflavone	Bilobetin	Ginkgetin	Quercetin	Bilobalide
COX-1	-6.2	-8.5	-10.6	-9.7	-9.7	-8.7	-7.5
COX-2	-8.8	-10.3	-11.9	-11.4	-11.2	-8.9	-8.3
LOX	-7.6	-8.7	-10.0	-9.8	-9.4	-9.0	-7.6

Receptor-ligand interactions

There are several properties in the interaction between biological receptors and their ligands (Specificity, affinity, saturation, physiological response, binding constant). In general, receptors bind tightly to a single natural ligand (specificity) and are distinguished from non-covalent interactions between molecules (affinity). Saturation refers to the ligand's concentration, while physiological response refers to the physiological response from receptor-ligand interaction, and the binding constant is the equilibrium constant in a chemical reaction. A competitive-binding assay is used to assess ligand specificity, while saturation can be assessed using a plot of concentration and total ligand (Attie & Raines, 2017). Appendix 2 presents the diagrammatic representations of the receptor-ligand interactions of all the docked complexes. Molecular interactions with amino acid residues, such as hydrogen bonds, van der Waals and electrostatic interactions, serve as indicators that the ligands are docked in favorable conformations (Hariono et al., 2016), among which hydrogen bonds and van der Waals are the most significant (Ferreira De Freitas & Schapira, 2017). The higher the number of interactions present, the stronger and the more stable the binding is.

In this study, all the ligands formed multiple interactions with the targeted proteins. The ligands from *G. biloba* were able to form a higher number of hydrogen bonds in comparison to the controls (Appendix 1). A high number of hydrogen bonds contributes to stronger binding, lowering the energy score and stabilizing the bonds (Tallei et al., 2020). Likewise, van der Waals interactions are formed in high numbers in the interactions between the receptors and the ligands of *G. biloba*. This type of interaction promotes the blocking of the target receptor by the ligand, despite the relatively weaker strength of these bonds compared to that of hydrogen bonds (Tallei et al., 2020). In addition, electrostatic interactions are observed in several receptor-ligand interactions. Electrostatic interactions are long-range interactions that are capable of leading binding partners to their appropriate binding positions (Mihiri Shashikala et al., 2019). Based on the analysis of receptor-ligand interactions, it can be inferred that in addition to displaying high binding affinities, the tested phytochemicals also formed stabilizing bonds with the protein targets.

Validation of experiment

The grid size and grid box center used were validated by re-docking the native ligands from each of the protein crystal structures against the three proteins selected. From this, it was found that these native ligands (FLC, NAG, 30Z) were able to bind to their respective proteins (COX-1, COX-2, LOX), hence generating various binding affinities of -5.8, -6.1 and -7.1 kcal/mol respectively, making it safe to suggest that the grid size and grid box center used were suitable (Table 5).

Table 5. Re-docking results for grid box validation

Protein	Native Ligand	Binding Affinity (kcal/mol)
COX-1	FLC (Citrate Anion)	-5.8
COX-2	NAG ($C_8H_{15}NO_6$)	-6.1
LOX	30Z ($C_{18}H_{22}O_4$)	-7.1

Lipinski's rule of five

To determine the potential of a compound to function as an oral drug, it is crucial for it to undergo drug-likeness evaluation. Lipinski's rule of five is the most widely used filter (no more than 5 hydrogen bond donors; no more than 10 hydrogen bond acceptors; molecular mass less than 500 Da; partition coefficient not greater than 5). The rule is used to assess drug-likeness as well as to determine the physicochemical features of an active substance that can be ingested orally in humans (Lipinski, 2004). In addition, it also

serves as a foundation for assessing the likelihood of success or failure of one compound with specific pharmacological or biological activity being developed as a drug. However, the rule must be used carefully to avoid the possible removal of promising compounds (Giménez et al., 2010).

The molecular weight, number of hydrogen bond acceptors, number of hydrogen bond donors, and lipophilicity (LogP) values for all the tested compounds are displayed in Table 6. This rule only allows one violation, as two or more violations may indicate a low solubility or permeability of the compound of interest in the human body (Benet et al., 2016). As shown in Table 7, all ligands meet Lipinski's rule of five, with amentoflavone as an exception showing two violations of the rule. Further study and probable chemical modifications would be needed to investigate the oral bioavailability of amentoflavone

Table 6. Molecular descriptors of Lipinski's Rule of Five

Ligands	Molecular Weight	LogP	No. of H-Bond Donor	No. of H-Bond Acceptor
Ginkgolide A	408.40 g/mol	0.6	2	9
Amentoflavone	538.46 g/mol	3.62	6	10
Bilobetin	552.48 g/mol	3.96	5	10
Ginkgetin	556.52 g/mol	4.34	4	10
Quercetin	302.24 g/mol	1.23	5	7
Bilobalide	326.30 g/mol	0.16	2	8

Table 7. Drug-Likeness of *G. biloba* Active Compounds

Ligands	No. of Violations	Drug-Likeness
Ginkgolide A	0	Yes
Amentoflavone	2	No
Bilobetin	1	Yes
Ginkgetin	1	Yes
Quercetin	0	Yes
Bilobalide	0	Yes

The potential use of the active compounds found in *G. biloba* as an alternative to NSAIDs indicated by the Molecular Docking studies conducted can be supported by their use in previous studies. A study conducted by Welton et al. (1986), reported in a review by Kim et al. (2004), found that quercetin was successful in serving as COX inhibitor from rat peritoneal macrophages. Another study performed by Ferrándiz et al. (1990) showed that quercetin was able to inhibit sheep platelet COX-1. Furthermore, ginkgetin was shown to downregulate COX-2 for both *in vivo* as well as *in vitro* studies when applied topically against skin inflammation without affecting the activity of COX-2 or COX-1 (Kwak et al., 2002). In addition to this, through *in vivo* and *in vitro* studies, ginkgolide A was found to be capable of inhibiting LPS-mediated inflammation through the downregulation of proinflammatory mediators, including COX-2 (Li et al., 2017). A

study conducted by Goldie & Dolan (2013) showed that bilobalide was able to inhibit inflammatory pain in rats upon oral administration and that no adverse effect was observed up to an administrate amount of 30 mg/kg, which complies with the findings obtained from the Lipinski's rule of five conducted in our study, in which bilobalide exhibited 0 violations (Table 7), and can thus be consumed orally. Kim et al. (1998) evaluated the anti-inflammatory and analgesic properties of amentoflavone, where it was administered intraperitoneally to lab mice and proved to exhibit a potent anti-inflammatory response. The use of an intraperitoneal method of administration in this study is in accordance with the results obtained from Lipinski's rule of five evaluation, where amentoflavone was shown to have two violations (Table 7) and, therefore, cannot be consumed orally. Bilobetin, on the other hand, has a relatively low content in nature; hence, reports on its metabolites are rare. Moreover, it has also been reported to possess potential kidney and liver toxicity (Feng et al., 2020), further explaining its use in scientific research. Although Lipinski's rule of five evaluation in this study suggests the drug-likeness of bilobetin (Table 7), further studies would be required to confirm the same.

Toxicity evaluation

Toxicity analysis was performed for the purpose of predicting the LD50 of each bioactive compound selected from *G. biloba* using the Pro Tox-II web server, from which it was found that quercetin and bilobalide were the most toxic, belonging to the toxicity class III, and an LD50 of 90 and 159 mg/kg respectively. Following this is ginkgolide A belonging to toxicity class IV, with an LD50 of 500 mg/kg. On the other hand, amentoflavone, bilobetin, and ginkgetin are the least toxic of all the compounds selected, belonging to class V toxicity, and an LD50 of 3919, 4000 and 4000 mg/kg, respectively. Table 8 shows the category of toxicity for each of the bioactive compounds selected. The median lethal dose (LD50) serves as a basis for categorizing chemical compounds with respect to their toxicities after acute exposure and refers to the lethal dose of a substance at which it is able to kill 50% of the population consuming the compound within 24 h of exposure to the same compound (Gadaleta et al., 2019). As per GHS (2011), there are five categories to classify the toxicity of a chemical; in which category I include chemicals with an LD50 ≤ 5 mg/kg, and category II covers all chemicals with an LD50 in the range of 5 < LD50 ≤ 50 mg/kg. Additionally, category III consists of all chemicals with an LD50 within the range of 50 < LD50 ≤ 300 mg/kg, whereas category IV includes chemicals with an LD50 in the range of 300 < LD50 ≤ 2000, and category V covers all chemicals with an LD50 >2000 mg/kg. This implies that out of all the compounds selected from *G. biloba*, quercetin and bilobalide were the most toxic, followed by ginkgolide A, amentoflavone, bilobetin, and ginkgetin.

In a study performed by Wang et al. (2021), Parkinson's disease (PD) was induced in mice through five days of treatment of 1 methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). For 26 days (including pretreatment), ginkgetin (5, 10, 20 mg/kg) and bilobalide (5, 10, 20 mg/kg) at different concentrations were orally administered to the PD mice. From the experiment, it was found that ginkgetin and bilobalide effectively improved the movements and muscle functions, enhanced neurotrophic, reduced oxidative damage, and activated microglial, thereby suggesting that ginkgetin can be consumed orally as an anti-inflammatory drug. Nevertheless, despite the greater toxicity of bilobalide in comparison with the other compounds selected from *G. biloba*, the study by Wang et al. (2020) suggests that when consumed orally at specific doses, it can be deemed to be safe. Furthermore, Cisplatin (CP) is a potent drug widely known for its antitumor activities. However, numerous side effects, particularly those that are toxic to the reproductive system, deter long-term use. In a study conducted by Negm et al. (2022), a single dose of CP (7 mg/kg IP) was shown to elicit testicular toxicity. Treatment with bilobetin (6 and 12 mg/kg orally for 10 days) was able to reduce the toxic effects. The protective effects of bilobetin against CP-induced testicular damage are linked to improved antioxidant effects, decreased apoptotic signals and the restoration of testes' ability to regenerate, thus suggesting the safety of bilobetin as an oral drug to replace NSAIDs. In the case of amentoflavone, the lower level of toxicity has led to its widespread use. For example, research by Su et al.

(2019) revealed that amentoflavone was able to regulate enzyme activity involved in glucose metabolism, increase insulin secretion as well as improve insulin signal transduction, particularly in targeted tissues. However, since amentoflavone violates two out of the five Lipinski rules (Table 7), it can be administered through alternative routes, such as intravenously or intramuscularly, instead of orally.

Table 8. Toxicity Classification of *G. biloba* Bioactive Compounds

Ligands	Toxicity Category
Ginkgolide A	Category IV
Amentoflavone	Category V
Bilobetin	Category V
Ginkgetin	Category V
Quercetin	Category III
Bilobalide	Category III

Limitations

The molecular docking used in this study was blind docking which indicates that no specific part of the enzyme was being targeted. In addition to that, there are still more compounds obtained from *G. biloba* that possess anti-inflammatory effects which were not included in this study, such as isoginkgetin and olivil 4-*O*-β-D-glucopyranoside that down-regulate the nitric oxide (NO), inducible NO synthase (iNOS), and tumour necrosis factor-α (TNF-α) (Li et al., 2019).

CONCLUSION

Binding energy values resulting from molecular docking studies showed that all the tested compounds could bind to their targets with high affinities. Further analysis of receptor-ligand interactions indicated that the tested compounds formed stable interactions with the proteins. Evaluation of the drug-likeness of the tested compounds using Lipinski's rule of five showed that the tested compounds (with the exception of amentoflavone) possessed the ability to be administered as oral drugs. It can thus be concluded that the tested phytocompounds of *G. biloba* could potentially serve as novel alternatives for conventional NSAIDs. Regardless of the several adverse effects found, *G. biloba* is considered safe for consumption and no significant adverse reaction or drug interaction has been found. Future wet lab drug-development experiments to verify our findings would be extremely useful in avoiding NSAIDs-caused indispositions. For further studies, systematic and sensitive assays should be developed in addition to the current validation test formats. It is also important to note that doses used in animal studies do not represent accepted doses for humans. Furthermore, further studies may consider using a targeted docking method and investigate more compounds from *G. biloba* that have anti-inflammatory properties, which may improve the potential utilization of *G. biloba* as a source of anti-inflammatory compounds.

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APPENDIX

Appendix 1a. Types and number of interactions formed between COX-1 and ligands

Protein	Ligand	Interaction										Total
		vdW	CH	HB	A/Pi-A	Pi-S	Pi-Sg	PP	Pi-cat/an	UDD/ UAA	H	
COX-1	Ibuprofen (control)	9	1	0	4	0	1	0	0	0	0	15
	Ginkgolide A	7	0	2	2	0	1	0	0	0	0	12
	Amentoflavone	8	0	5	2	1	1	1	6	1	0	25
	Bilobetin	14	1	1	2	0	0	1	3	0	0	22
	Ginkgetin	13	0	2	3	0	2	1	2	0	0	23
	Quercetin	10	1	2	5	1	0	1	0	1	0	21
	Bilobalide	6	0	3	2	0	0	0	0	0	0	11

Appendix 1b. Types and number of interactions formed between COX-2 and ligands

Protein	Ligand	Interaction										Total
		vdW	CH	HB	A/Pi-A	Pi-S	Pi-Sg	PP	Pi-cat/an	UDD/ UAA	H	
COX-2	Celecoxib (control)	8	2	3	1	1	1	0	0	1	3	20
	Ginkgolide A	13	0	4	1	0	0	0	0	1	0	19
	Amentoflavone	15	0	3	3	0	0	2	2	1	0	26
	Bilobetin	14	0	3	3	0	0	1	1	1	0	23
	Ginkgetin	11	2	4	3	0	0	1	1	1	0	23
	Quercetin	8	0	4	5	1	0	0	0	2	0	20
	Bilobalide	13	0	1	0	0	0	0	0	0	0	14

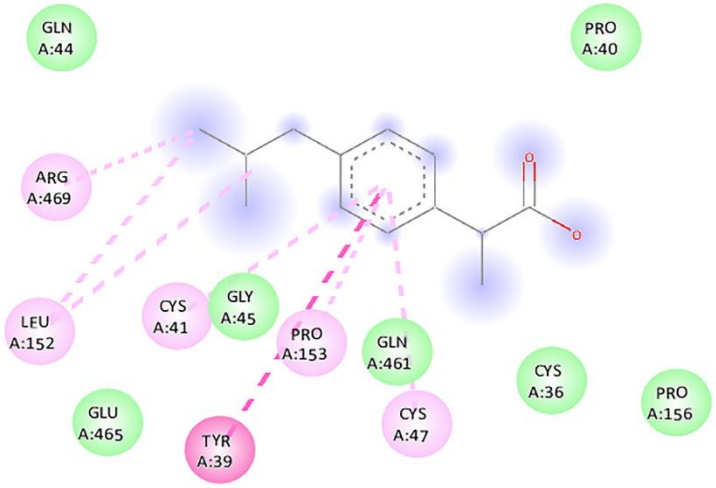
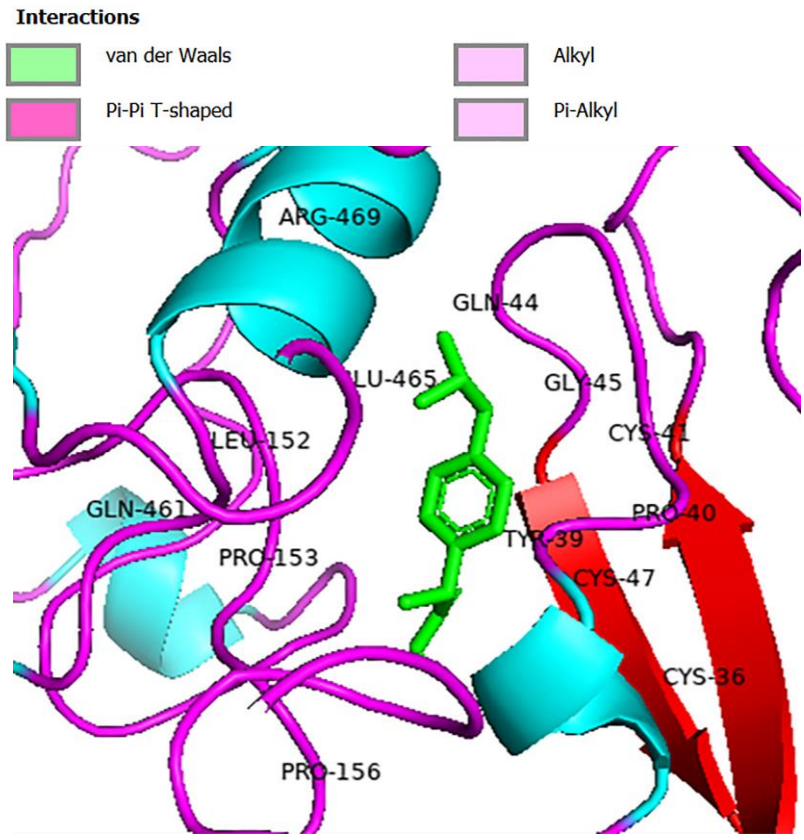
Appendix 1c. Types and number of interactions formed between LOX and ligands

Protein	Ligand	Interaction										Total
		vdW	CH	HB	A/Pi-A	Pi-S	Pi-Sg	PP	Pi-cat/an	UDD/UAA	H	
LOX	Benzydamine (control)	9	2	0	5	0	0	0	2	0	0	18
	Ginkgolide A	10	1	3	0	0	0	0	0	0	0	14
	Amentoflavone	12	0	2	5	0	3	2	1	0	0	25
	Bilobetin	10	2	2	2	0	0	0	4	1	0	21
	Ginkgetin	8	0	2	4	0	3	4	0	0	0	21

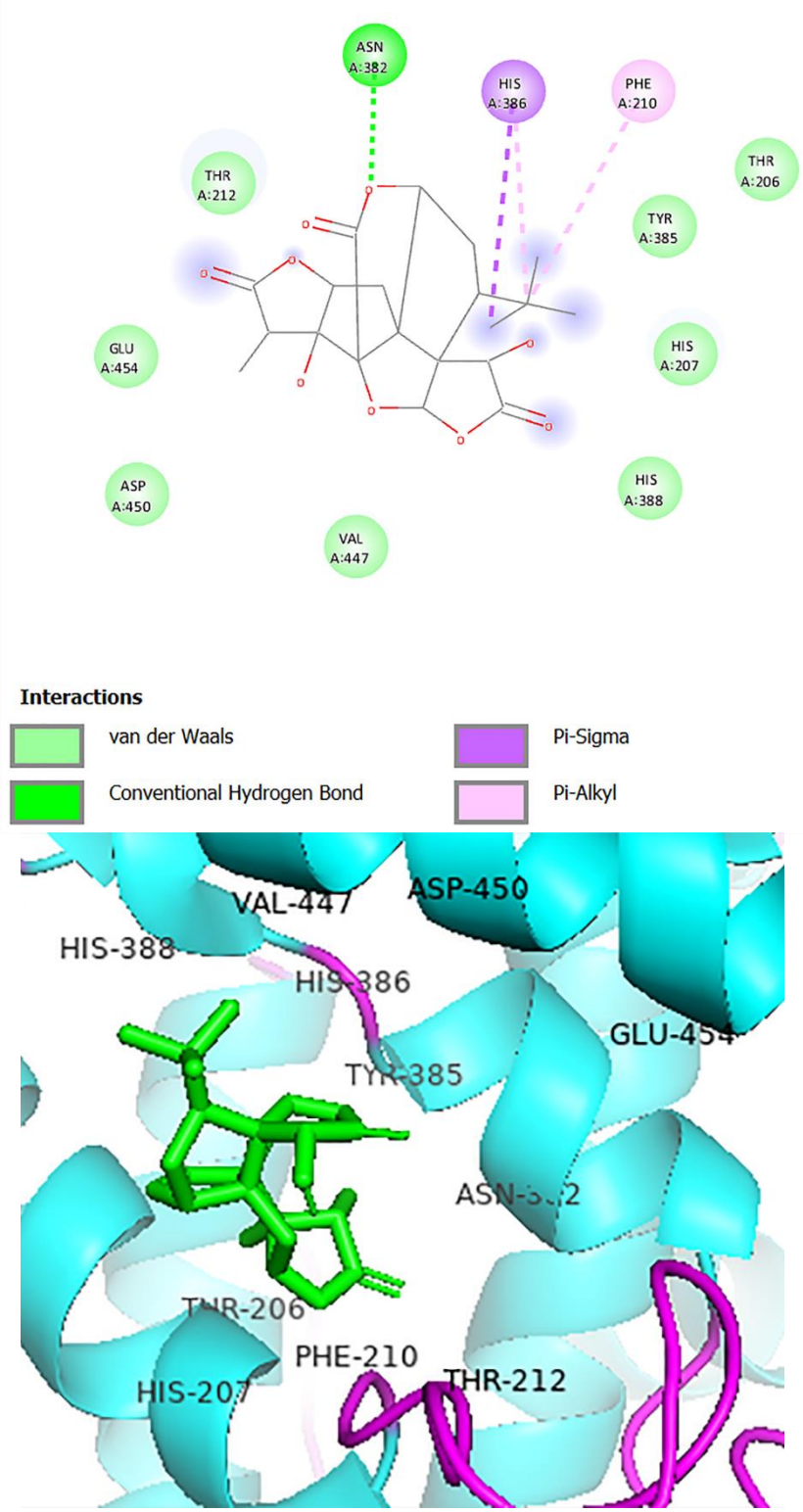
	Quercetin	14	0	5	4	0	0	0	1	0	0	24
	Bilobalide	9	2	3	0	0	0	0	0	0	0	14

(vdW: van der Waals; CH: carbon-hydrogen bond; HB: hydrogen bond; A/Pi-A: alkyl/pi-alkyl; Pi-S: pi-sulfur; Pi-Sg: pi-sigma; PP: pi-pi interaction; Pi-cat/an: pi-cation/anion; UDD/UAA: unfavorable donor-donor/unfavorable acceptor-acceptor; H: halogen)

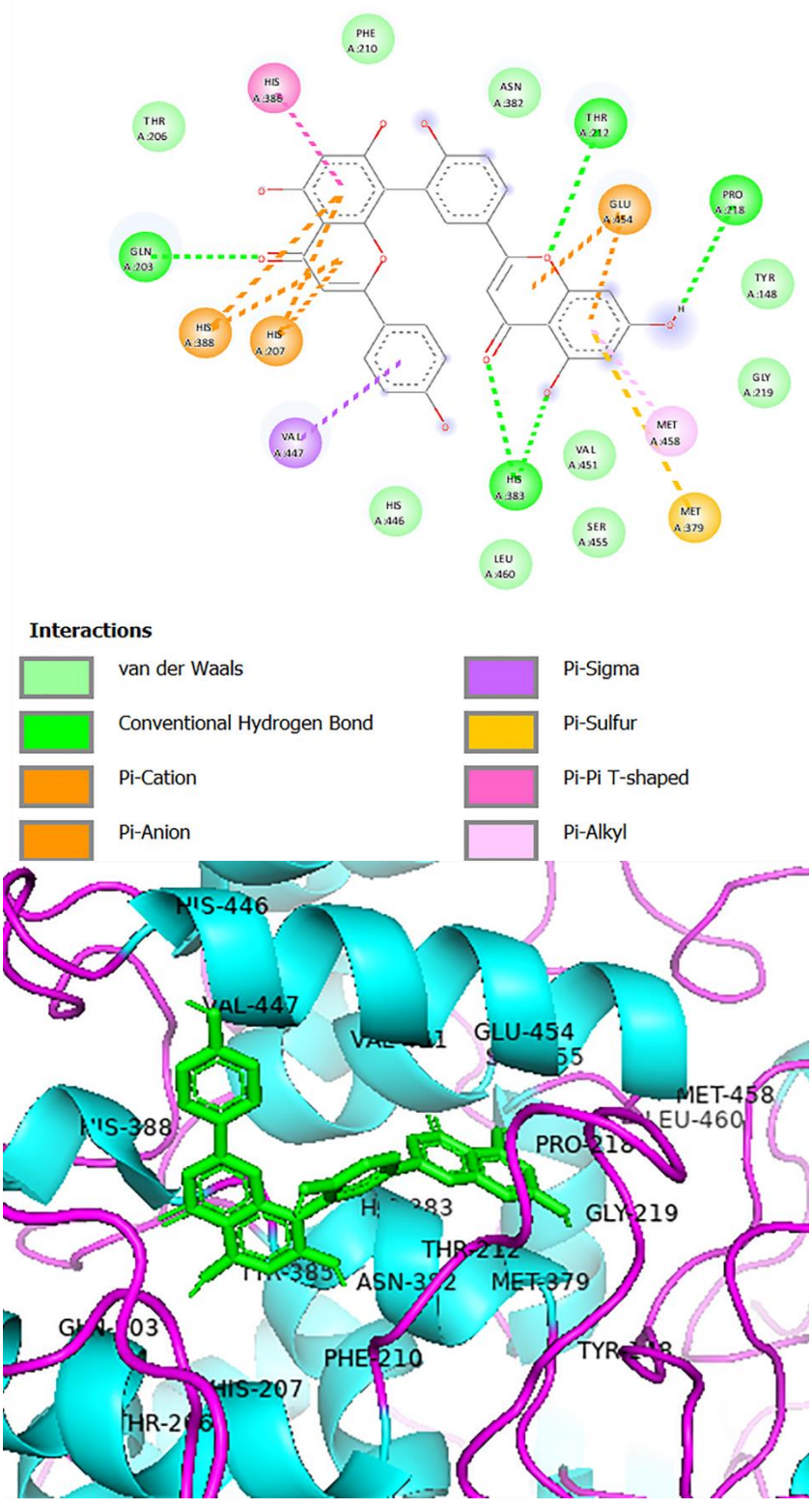
Appendix 2. Receptor-ligand interactions between controls and *Ginkgo biloba* compounds and protein targets

Protein	Ligand	Receptor-ligand complex
COX-1	Control (Ibuprofen)	
		

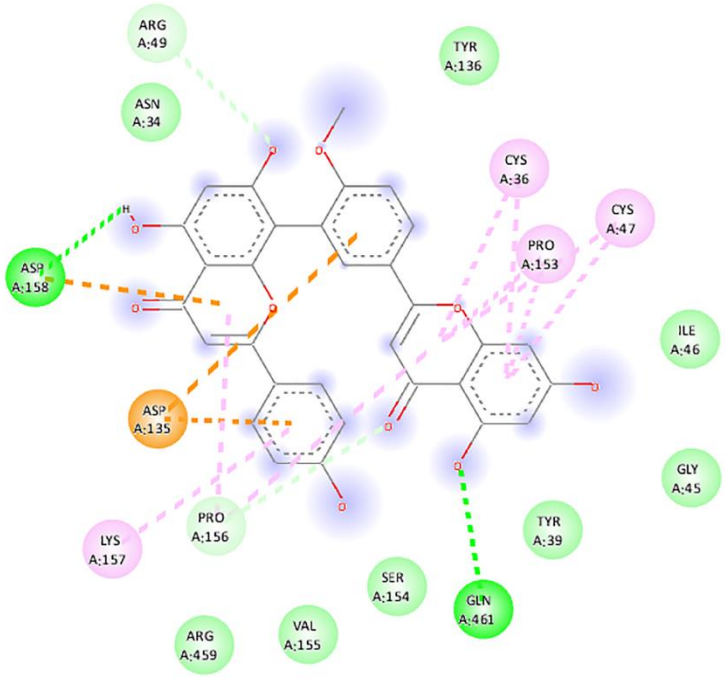
Ginkgolide A



Amentoflavone



Bilobetin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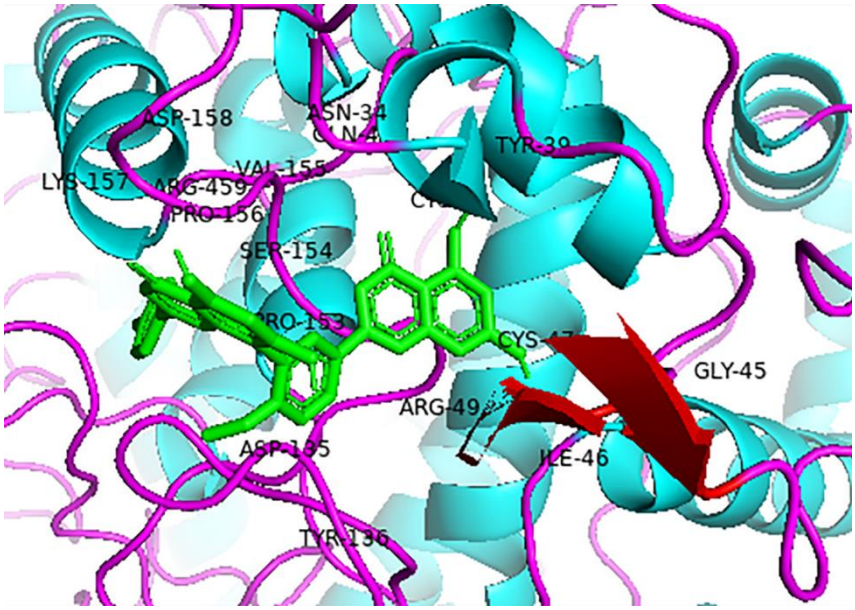
TYR A:136
ASN A:34
CYS A:36
CYS A:47
PRO A:153
ASP A:158
ILE A:46
ASP A:135
GLY A:45
PRO A:156
TYR A:39
LYS A:157
SER A:154
GLN A:461
ARG A:459
VAL A:155


Interactions
van der Waals
Pi-Anion
Conventional Hydrogen Bond
Pi-Alkyl
Carbon Hydrogen Bond


ASP-158
ASN-34
TYR-39
LYS-157
VAL-155
ARG-459
PRO-156
SER-154
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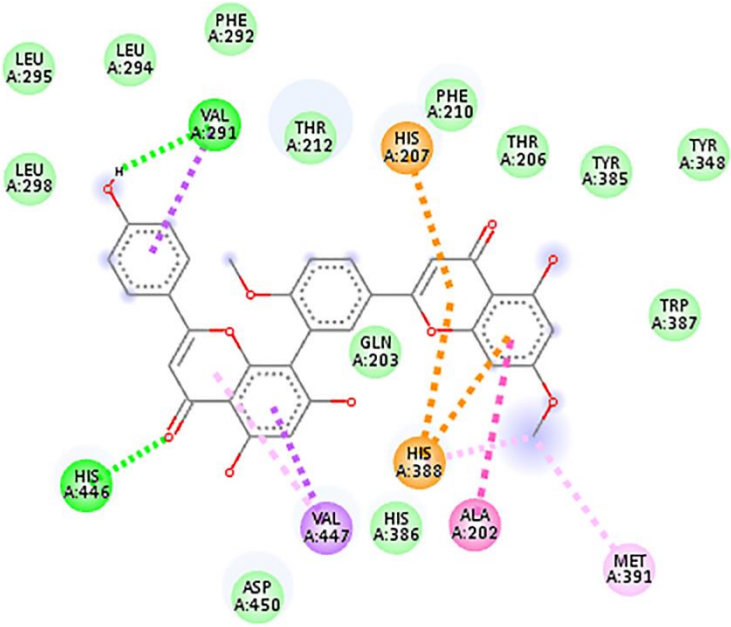
CYS-47
GLY-45
ARG-49
ASP-135
ILE-46
TYR-136

Ginkgetin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LEU A:295
LEU A:294
VAL A:291
THR A:212
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HIS A:207
THR A:206
TYR A:385
TYR A:348
LEU A:298
TRP A:387
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HIS A:388
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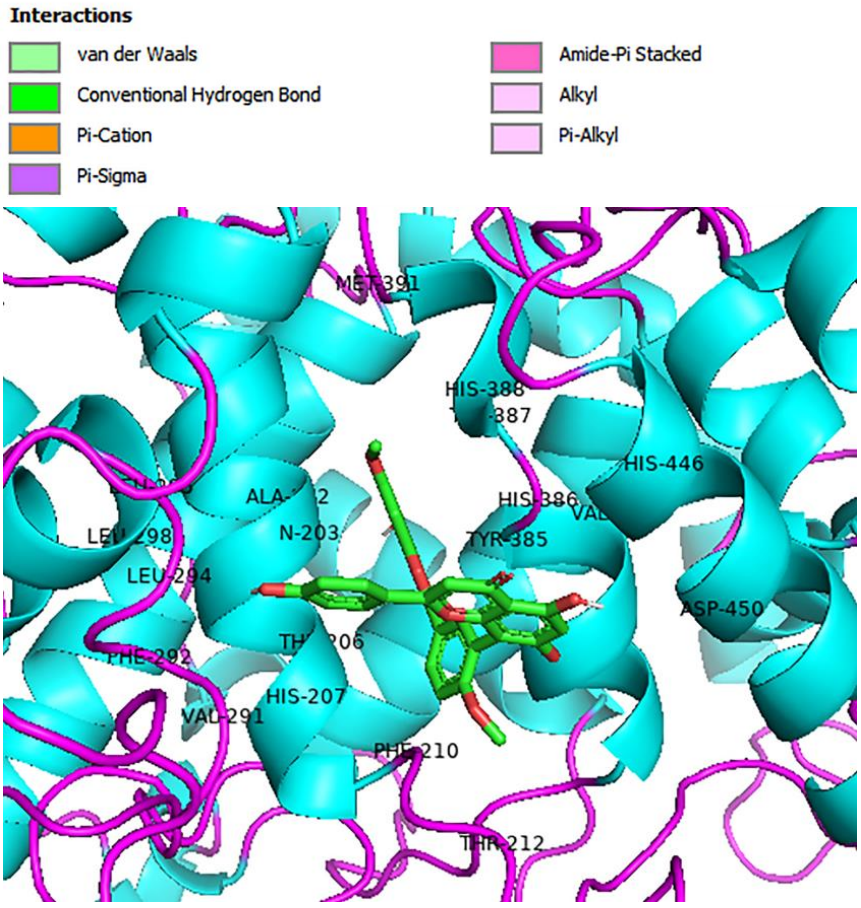
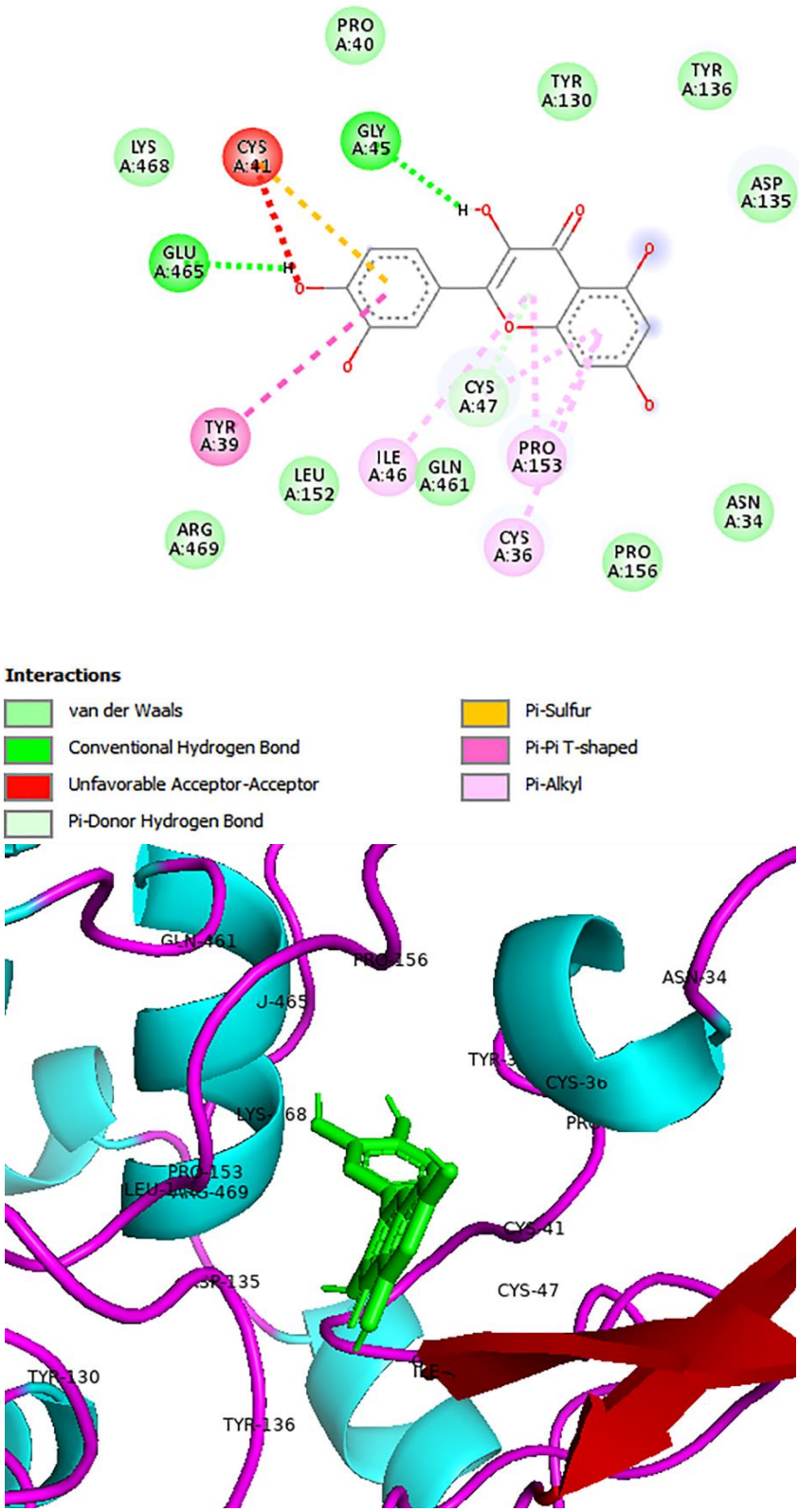
ALA A:202
MET A:391
ASP A:450


Interactions
van der Waals
Conventional Hydrogen Bond
Pi-Cation
Pi-Sigma
Amide-Pi Stacked
Alkyl
Pi-Alkyl
MET-391
HIS-388
HIS-446
TYR-385
LEU-294
ASP-450
PHE-292
HIS-207
VAL-291
PHE-210
THR-212

Quercetin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LYS A:468
CYS A:41
GLY A:45
ASP A:135
GLU A:465
CYS A:47
TYR A:39
ILE A:46
GLN A:461
PRO A:153
LEU A:152
ASN A:34
ARG A:469
CYS A:36
PRO A:156
Interactions
van der Waals
Conventional Hydrogen Bond
Unfavorable Acceptor-Acceptor
Pi-Donor Hydrogen Bond
Pi-Sulfur
Pi-Pi T-shaped
Pi-Alkyl
GLN-461
PRO-156
ASN-34
CYS-36
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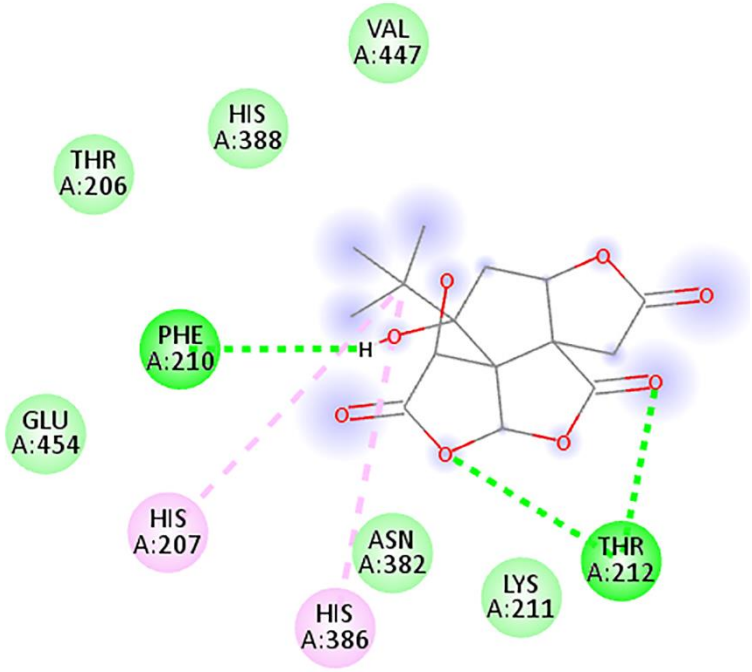
CYS-41
CYS-47
TYR-130
TYR-136

Bilobalide


VAL
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HIS
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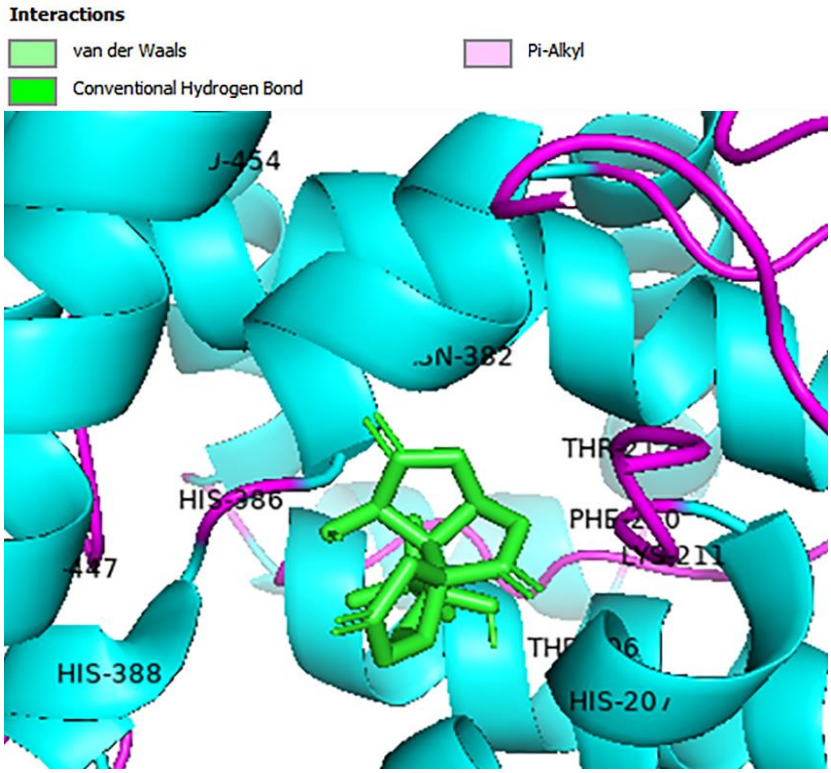
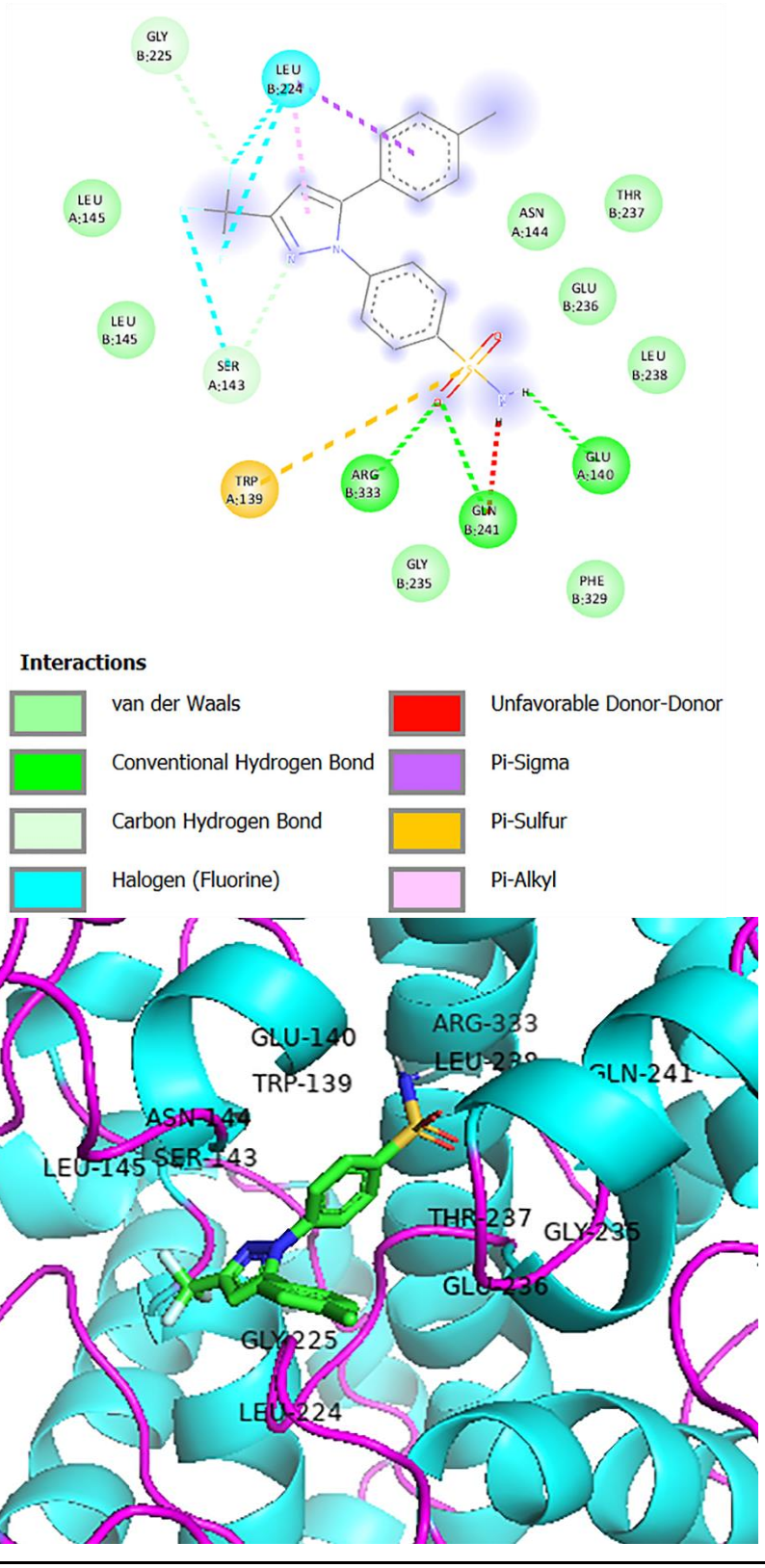
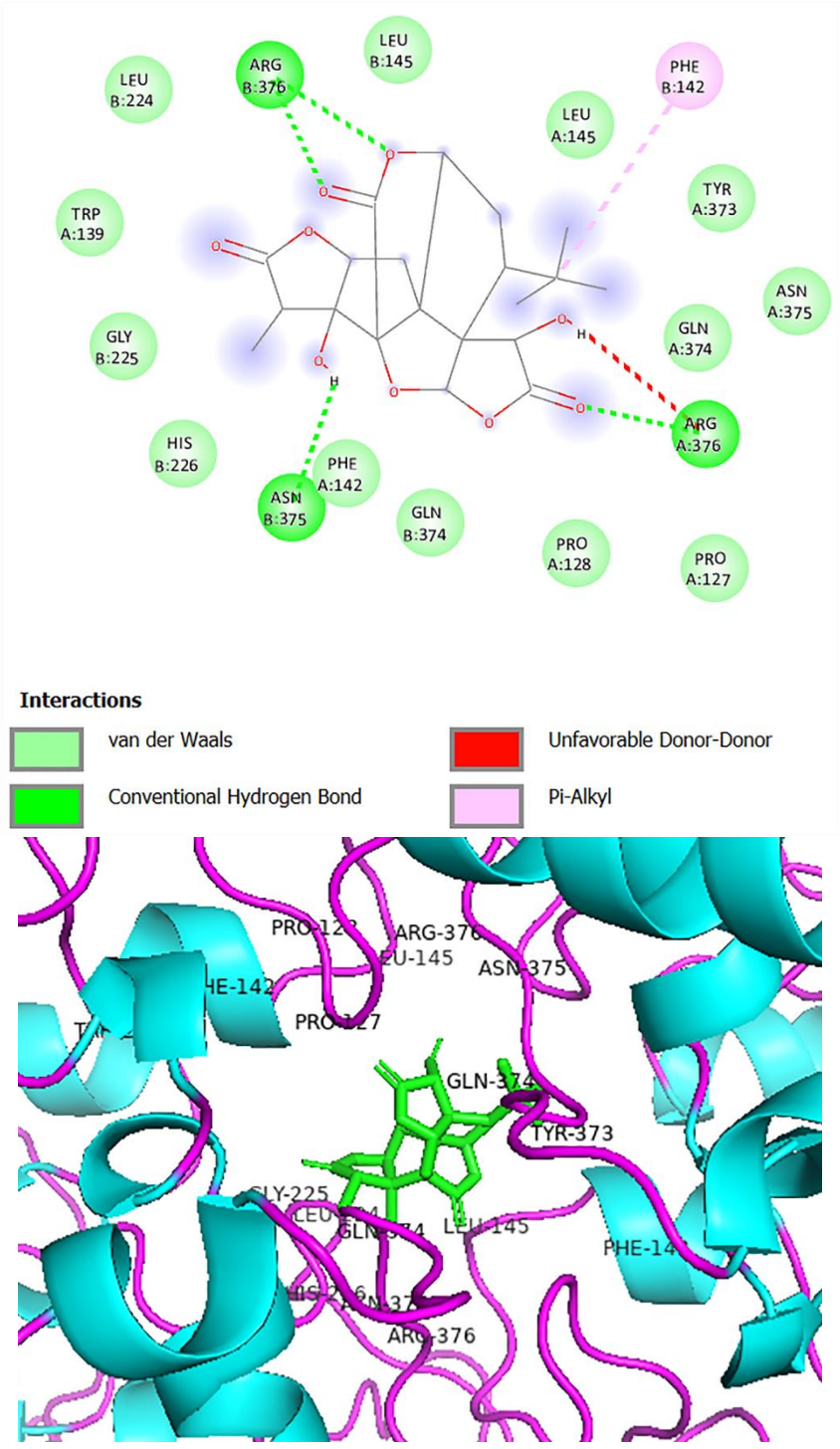
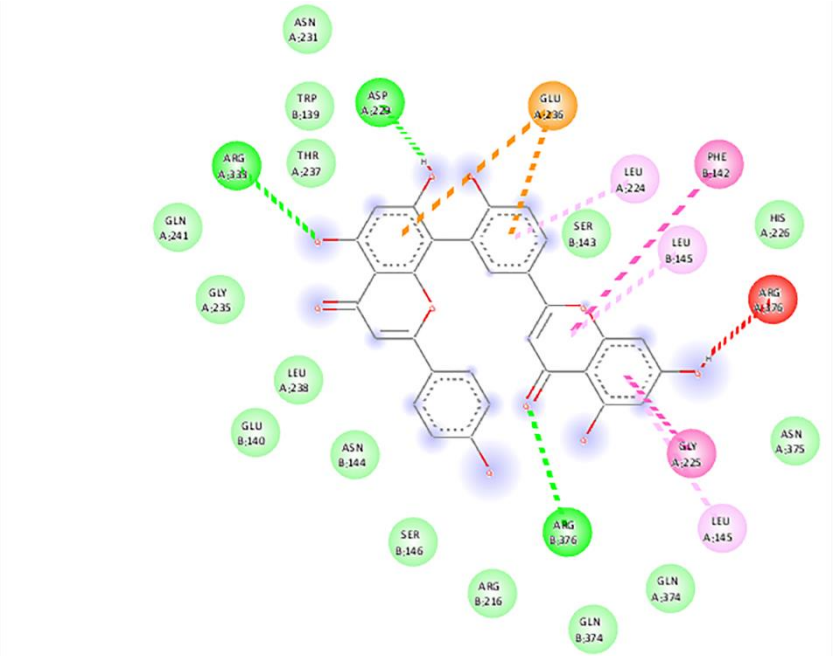

Interactions
van der Waals
Pi-Alkyl
Conventional Hydrogen Bond
HIS-386
HIS-388

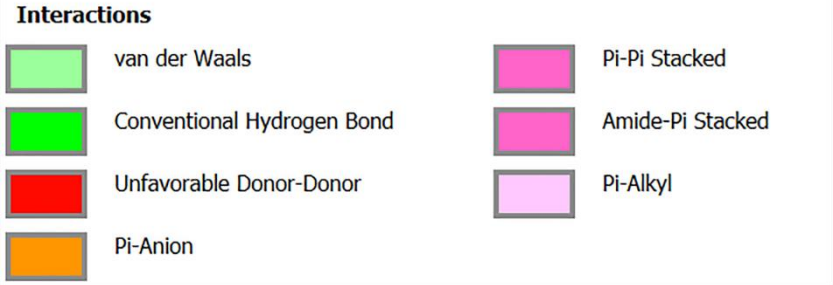
COX-2	Control (Celecoxib)	

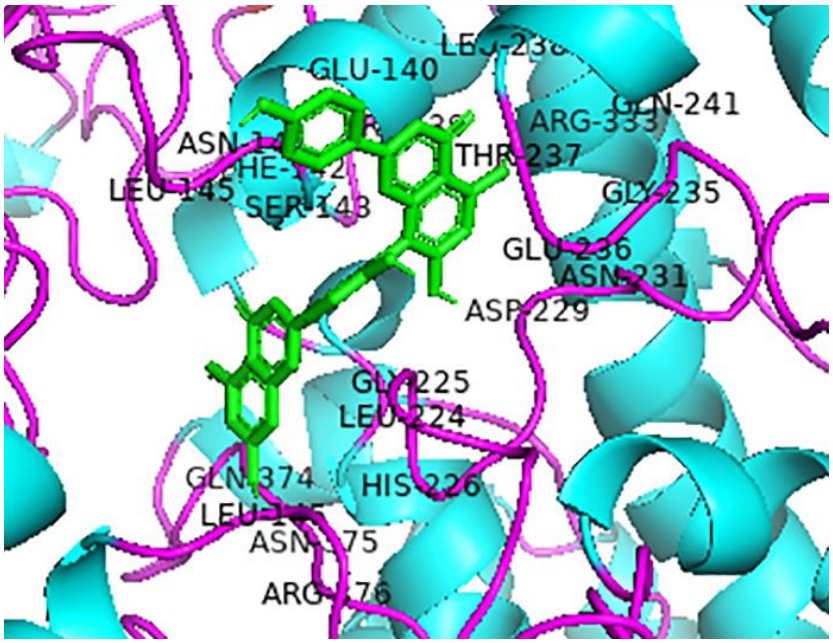
Ginkgolide A



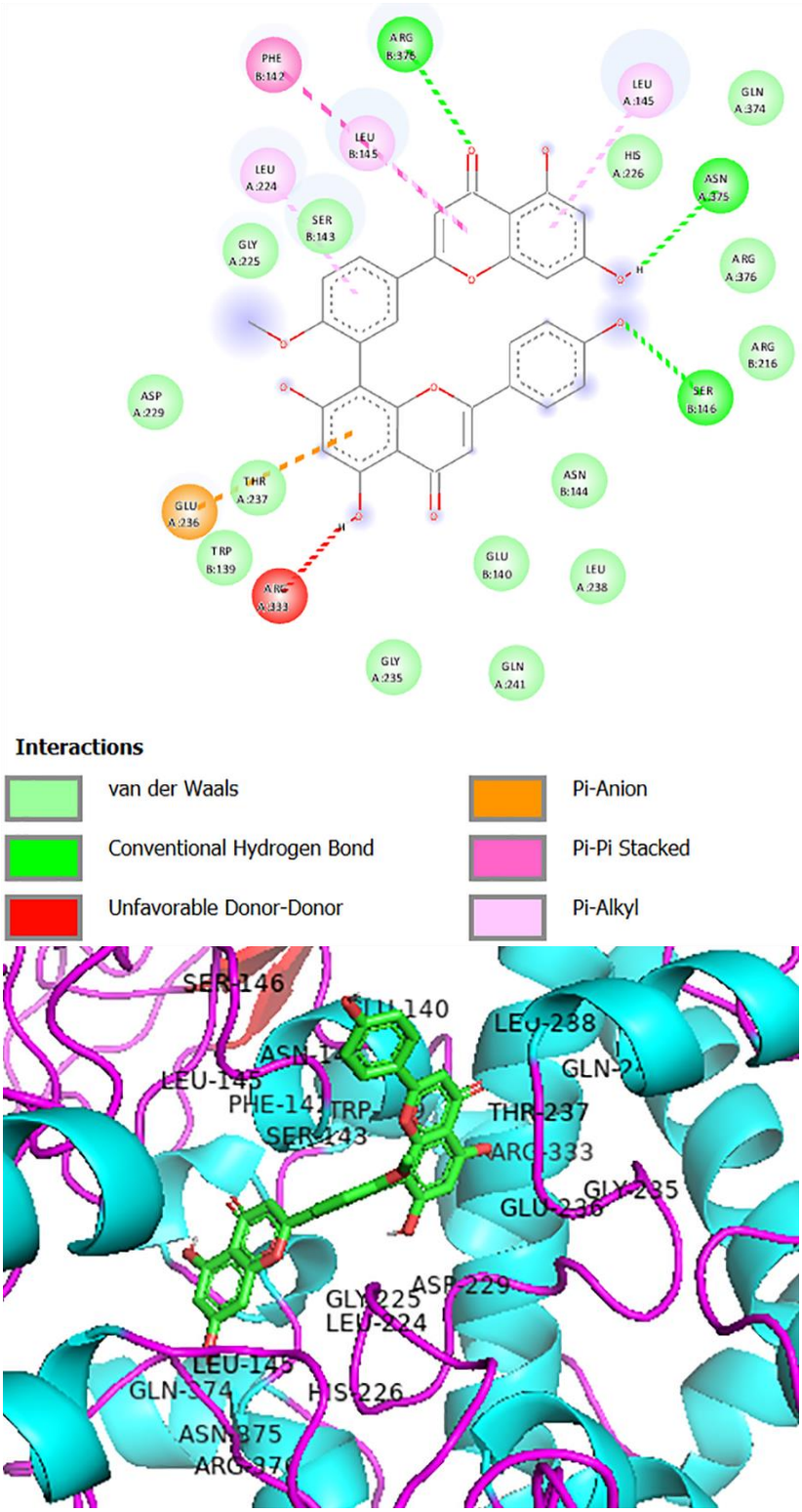
Amentoflavone







Bilobetin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GLY A:225
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ARG B:216
ASP A:229
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ASN B:144
THR A:237
GLU A:236
TRP B:139
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ARG A:333
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Interactions
van der Waals
Pi-Anion
Conventional Hydrogen Bond
Pi-Pi Stacked
Unfavorable Donor-Donor
Pi-Alkyl
SER-146
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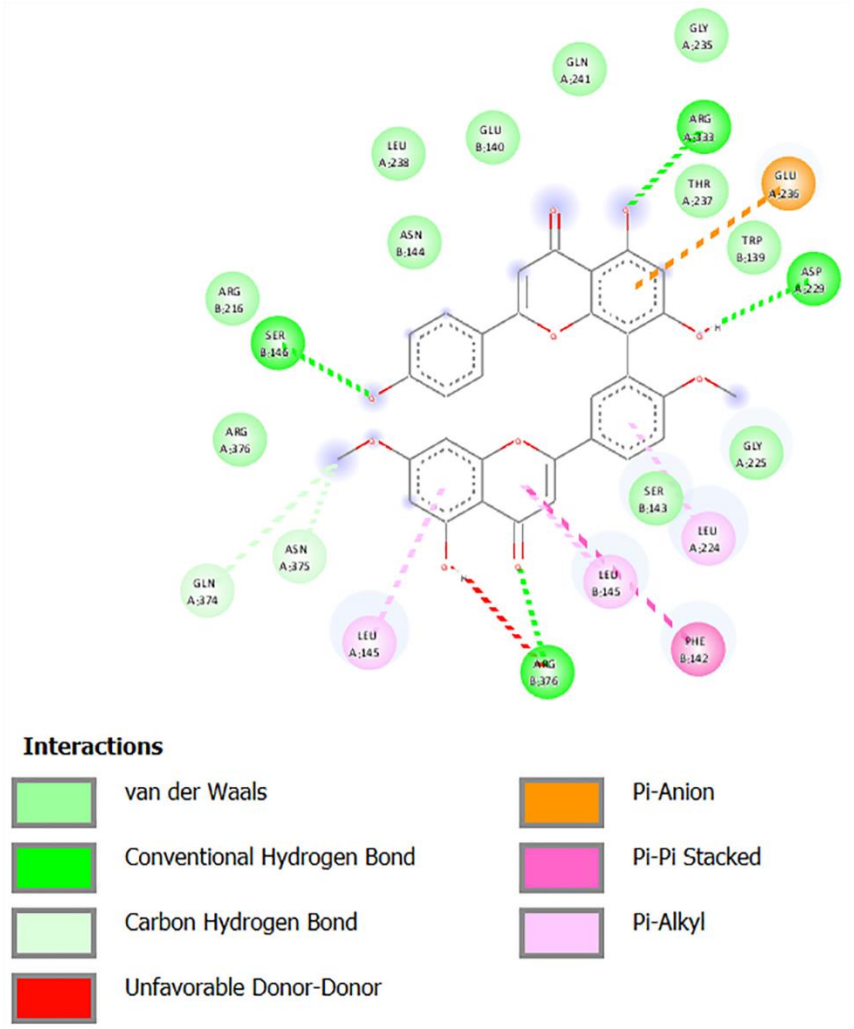
GLY-225
LEU-224
LEU-145
GLN-374
HIS-226
ASN-375

Ginkgetin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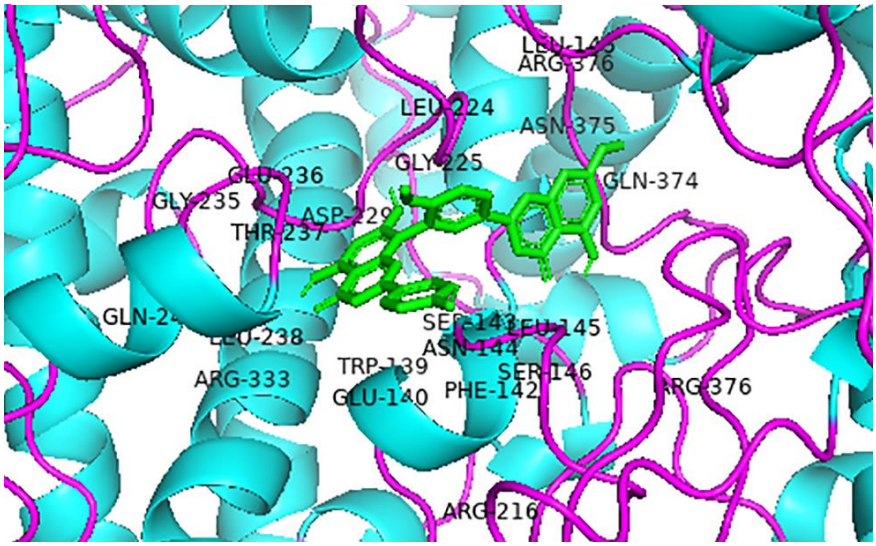
GLN A:241
ARG A:333
LEU A:238
GLU B:140
THR A:237
GLU A:236
ASN B:144
TRP B:139
ASP A:229
ARG B:216
SER B:146
ARG A:376
GLY A:225
SER B:143
LEU A:224
ASN A:375
LEU B:145
GLN A:374
LEU A:145
PHE B:142
ARG B:376
Interactions
van der Waals
Pi-Anion
Conventional Hydrogen Bond
Pi-Pi Stacked
Carbon Hydrogen Bond
Pi-Alkyl
Unfavorable Donor-Donor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LEU-224
ASN-375
GLY-225
GLU-236
GLN-374
GLY-235
ASP-229
THR-237
SER-143
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TRP-139
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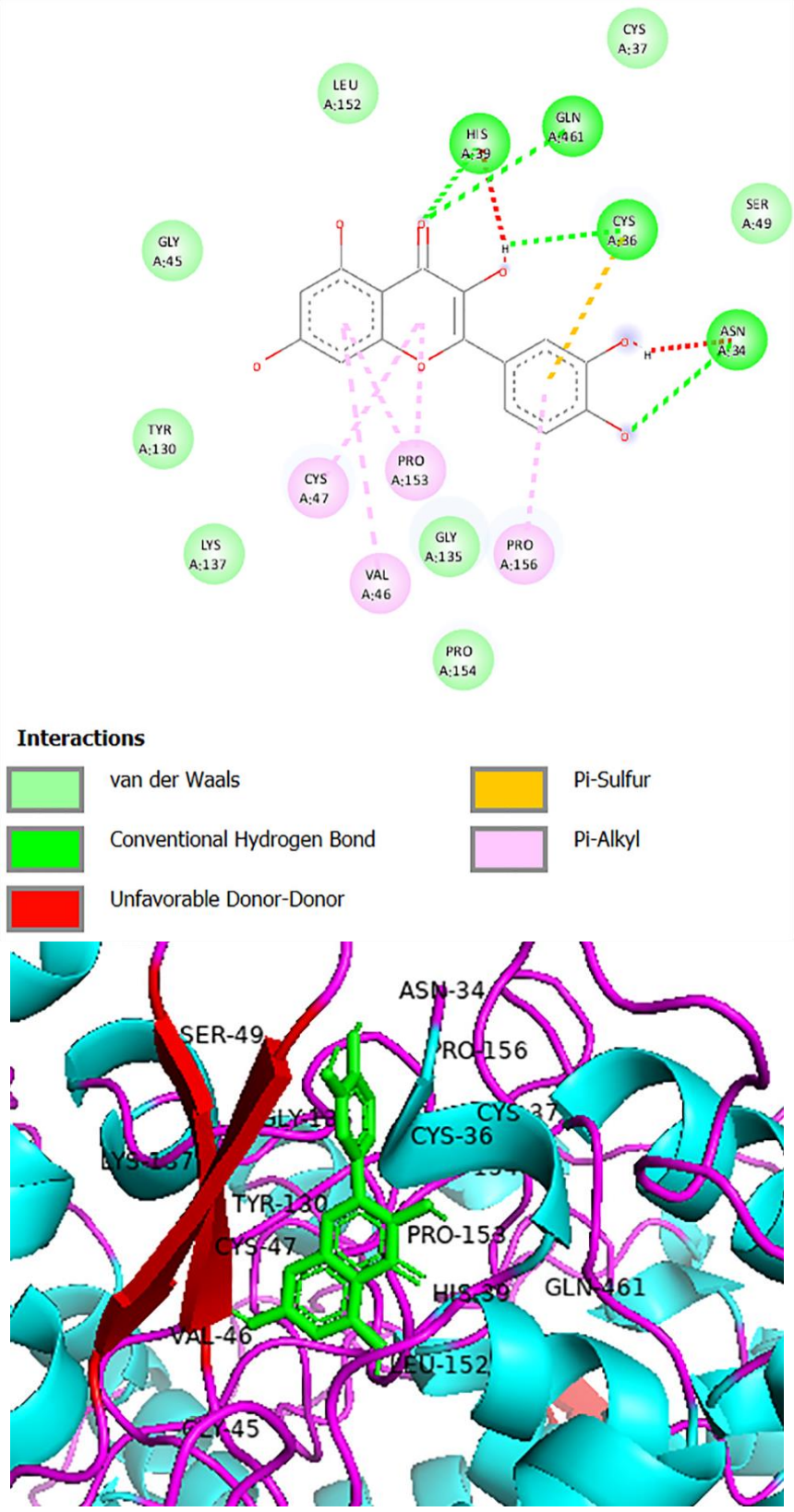
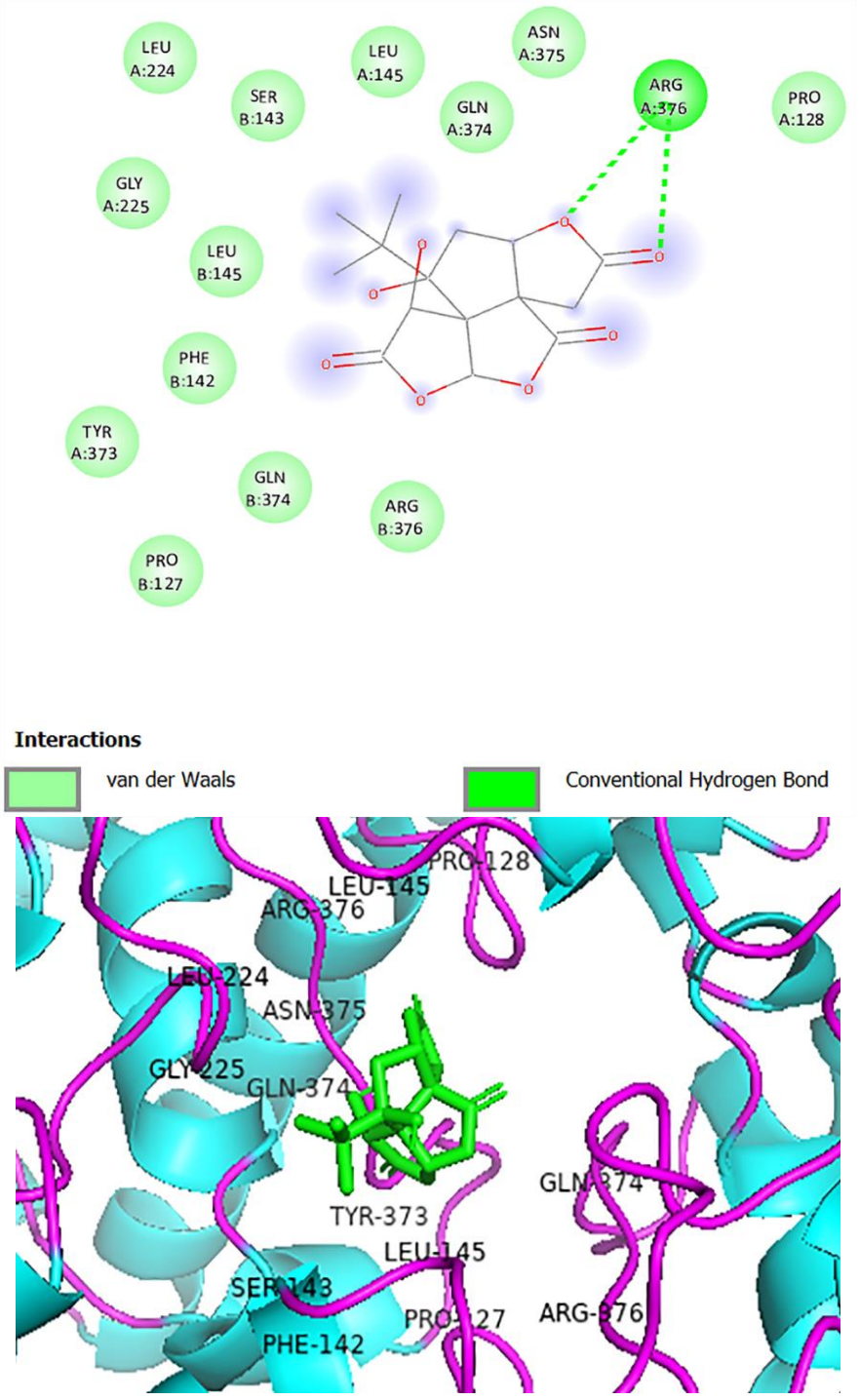
ARG-333
GLU-140
PHE-142
ARG-376
ARG-216

Quercetin


CYS A:37
LEU A:152
GLN A:461
HIS A:39
SER A:49
CYS A:36
GLY A:45
ASN A:34
TYR A:130
PRO A:153
CYS A:47
GLY A:135
PRO A:156
LYS A:137
VAL A:46
PRO A:154
Interactions
van der Waals
Pi-Sulfur
Conventional Hydrogen Bond
Pi-Alkyl
Unfavorable Donor-Donor
ASN-34
SER-49
PRO-156
CYS-36
TYR-130
PRO-153
CYS-47
GLN-461
VAL-46
LEU-152

Bilobalide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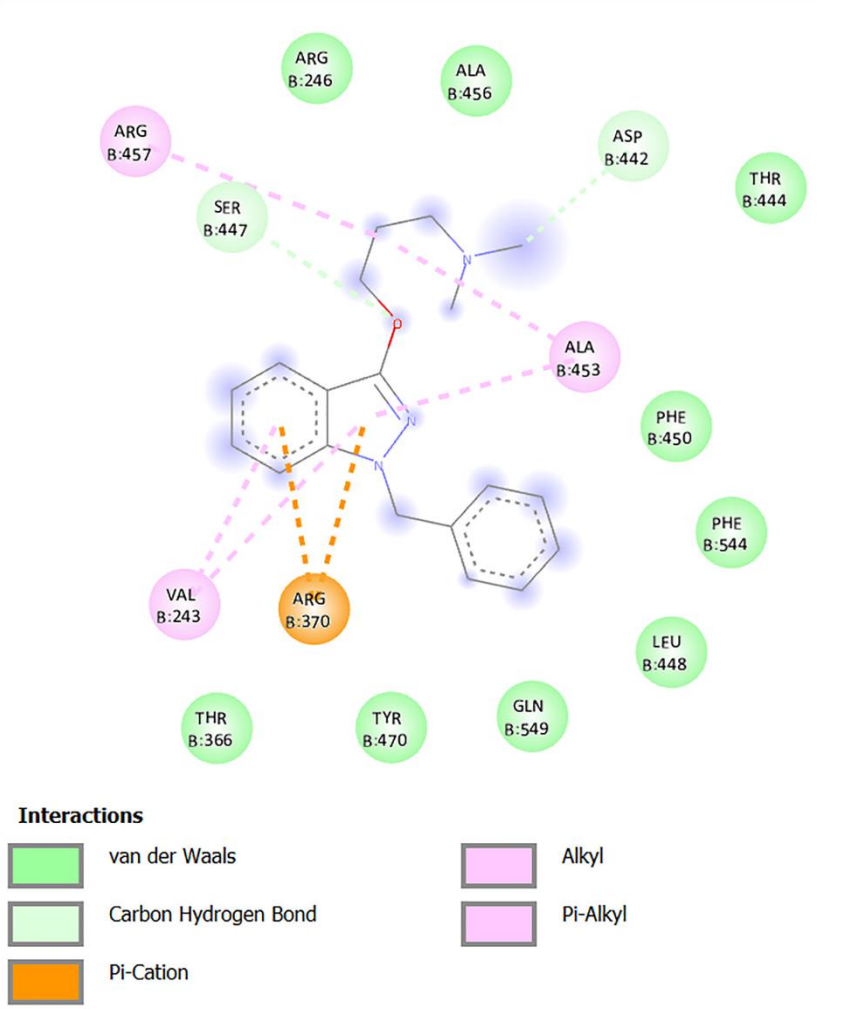
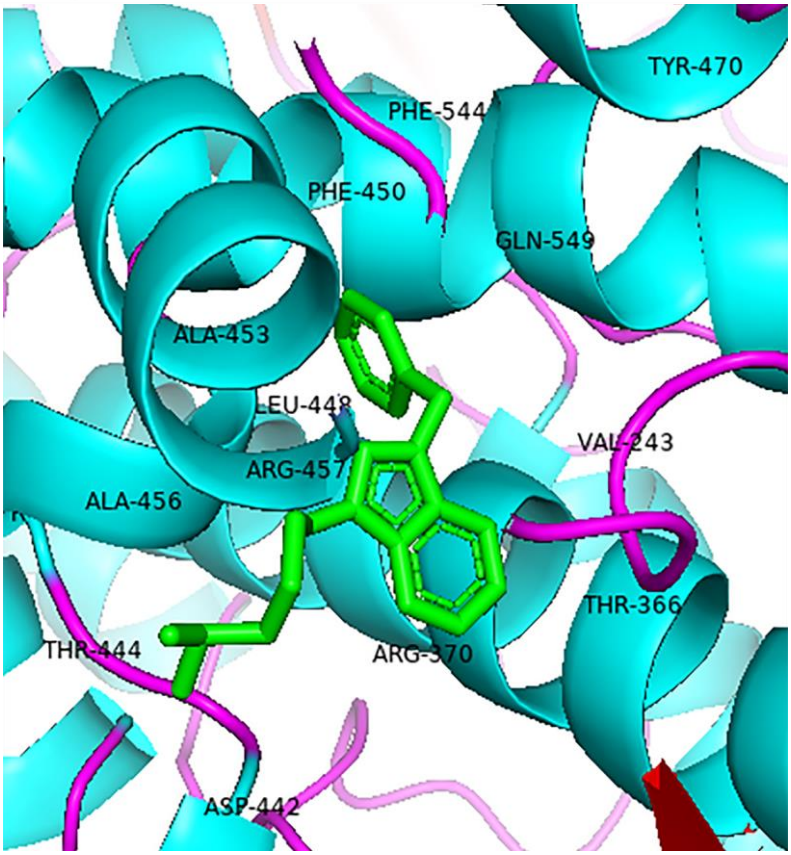
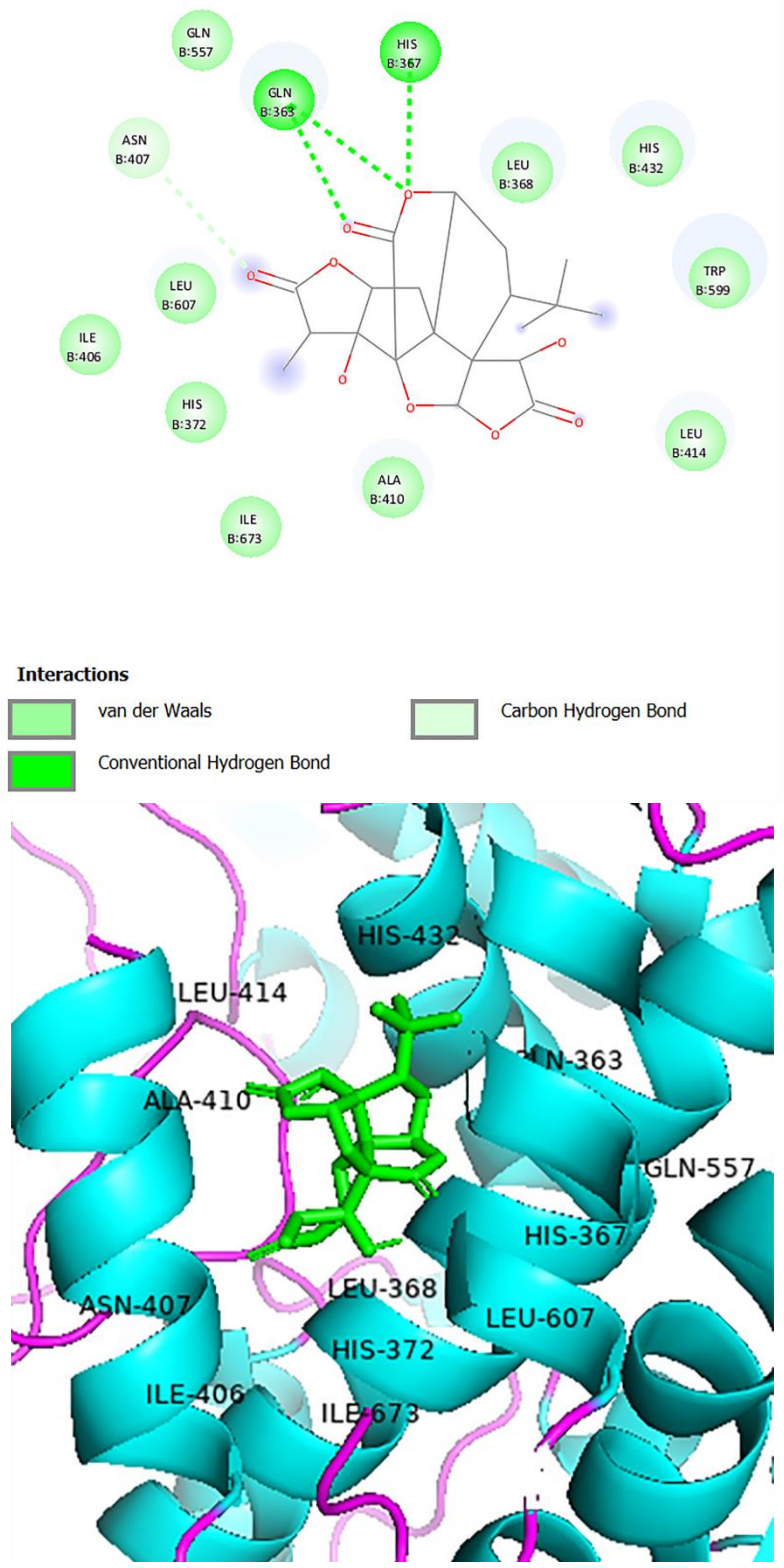
SER B:143
LEU A:145
ASN A:375
GLN A:374
ARG A:376
PRO A:128
GLY A:225
LEU B:145
PHE B:142
TYR A:373
GLN B:374
ARG B:376
PRO B:127
Interactions
van der Waals
Conventional Hydrogen Bond
PRO-128
LEU-145
ARG-376
LEU-224
ASN-375
GLY-225
GLN-374
TYR-373
LEU-145
SER-143
PHE-142
ARG-376

LOX Control (Benzydamine)

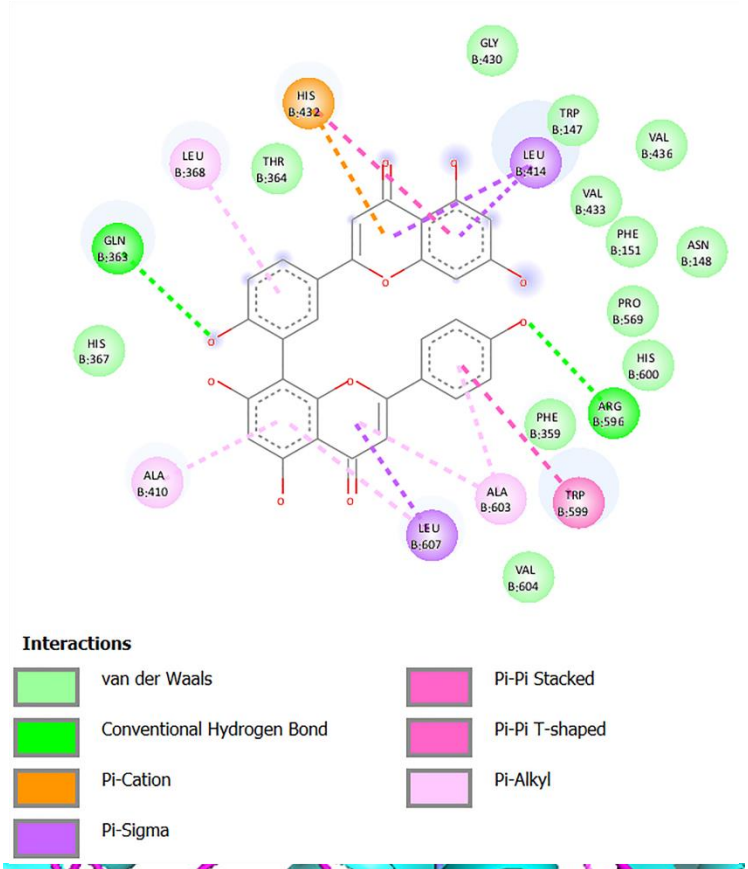


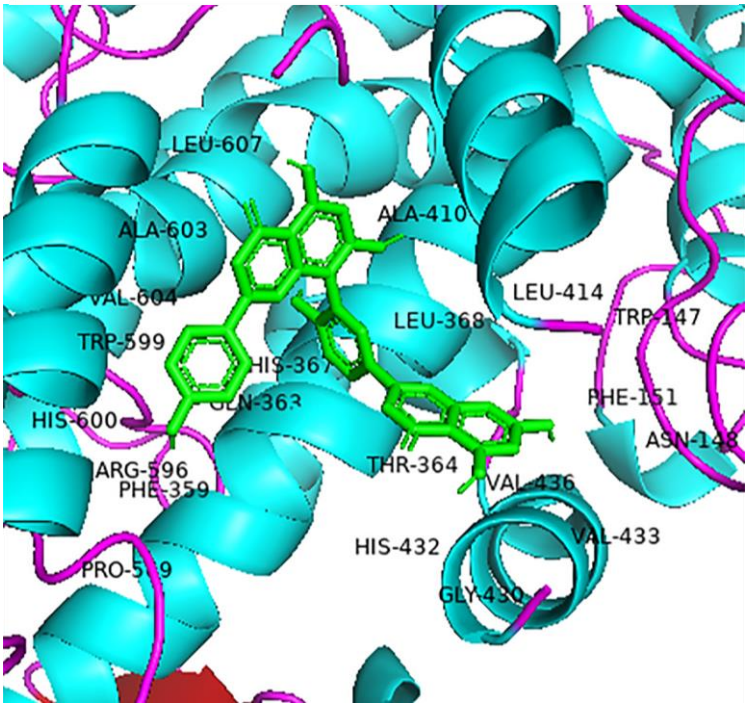


Ginkgolide A

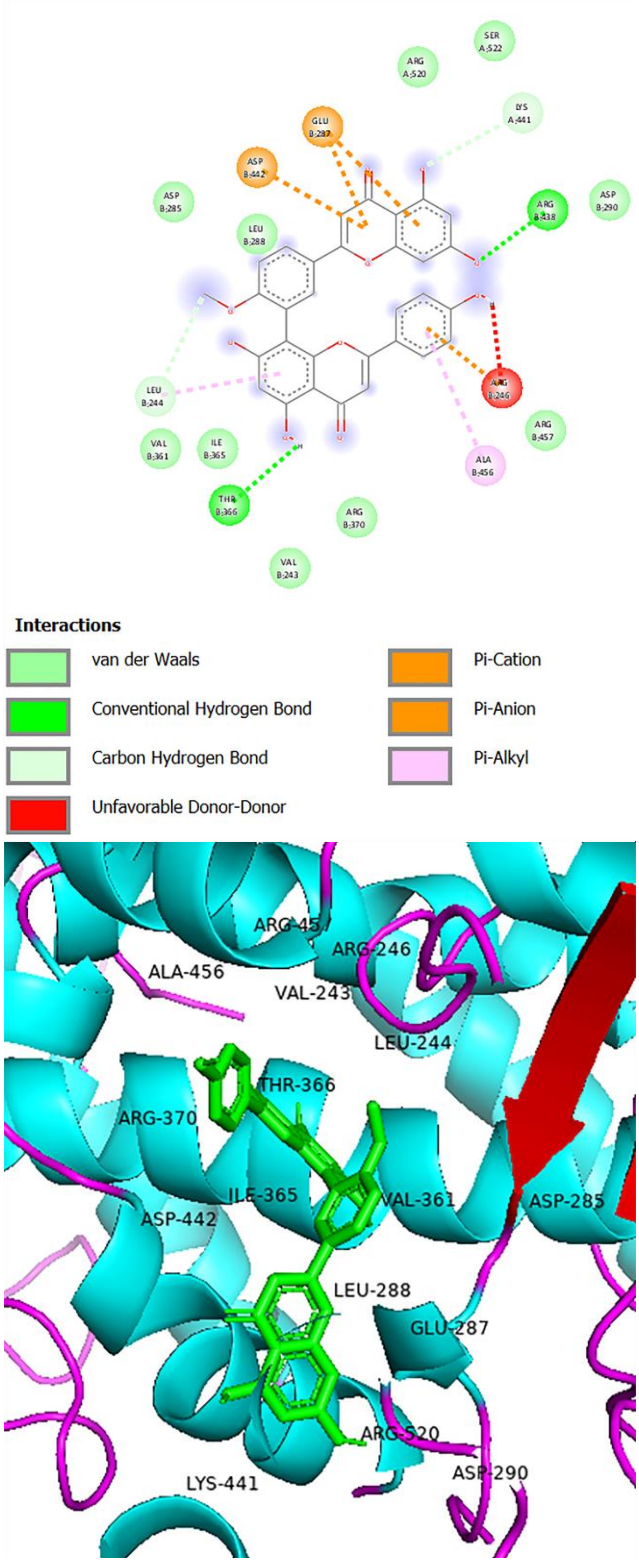


Amentoflavone

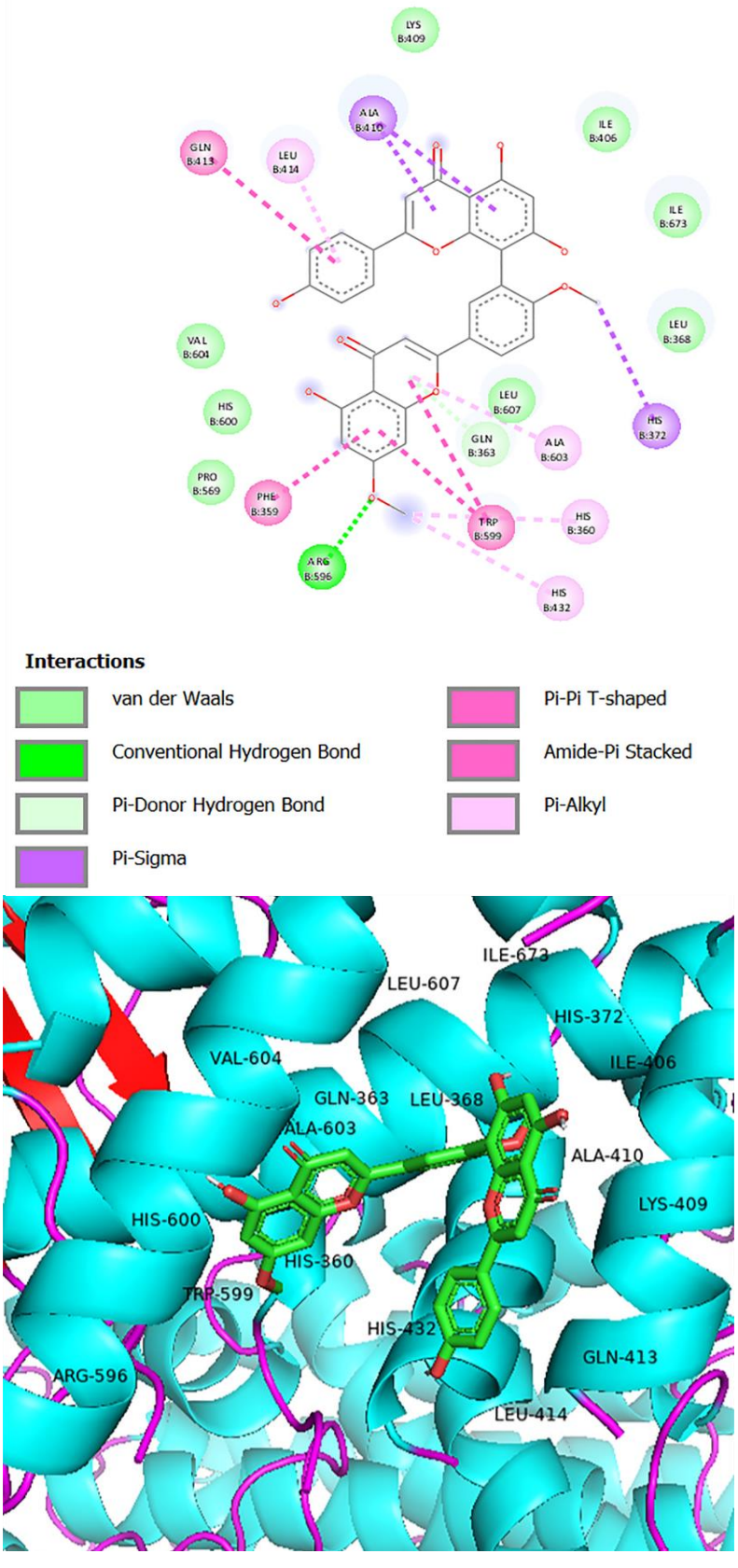




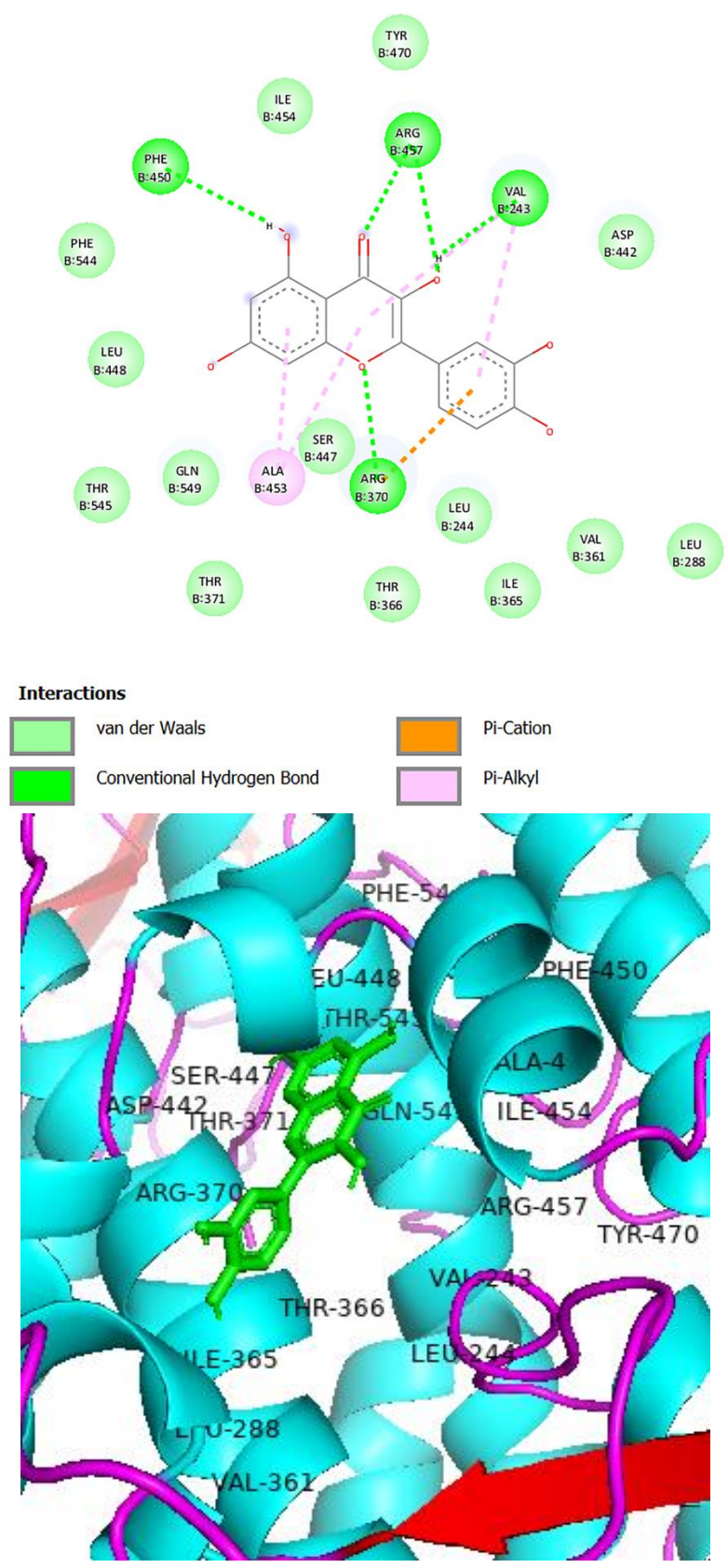
Bilobetin


Interactions
van der Waals
Conventional Hydrogen Bond
Carbon Hydrogen Bond
Unfavorable Donor-Donor
Pi-Cation
Pi-Anion
Pi-Alkyl
ARG-457
ARG-246
ALA-456
VAL-243
LEU-244
THR-366
ARG-370
ILE-365
VAL-361
ASP-285
ASP-442
LEU-288
GLU-287
ARG-520
ASP-290
LYS-441

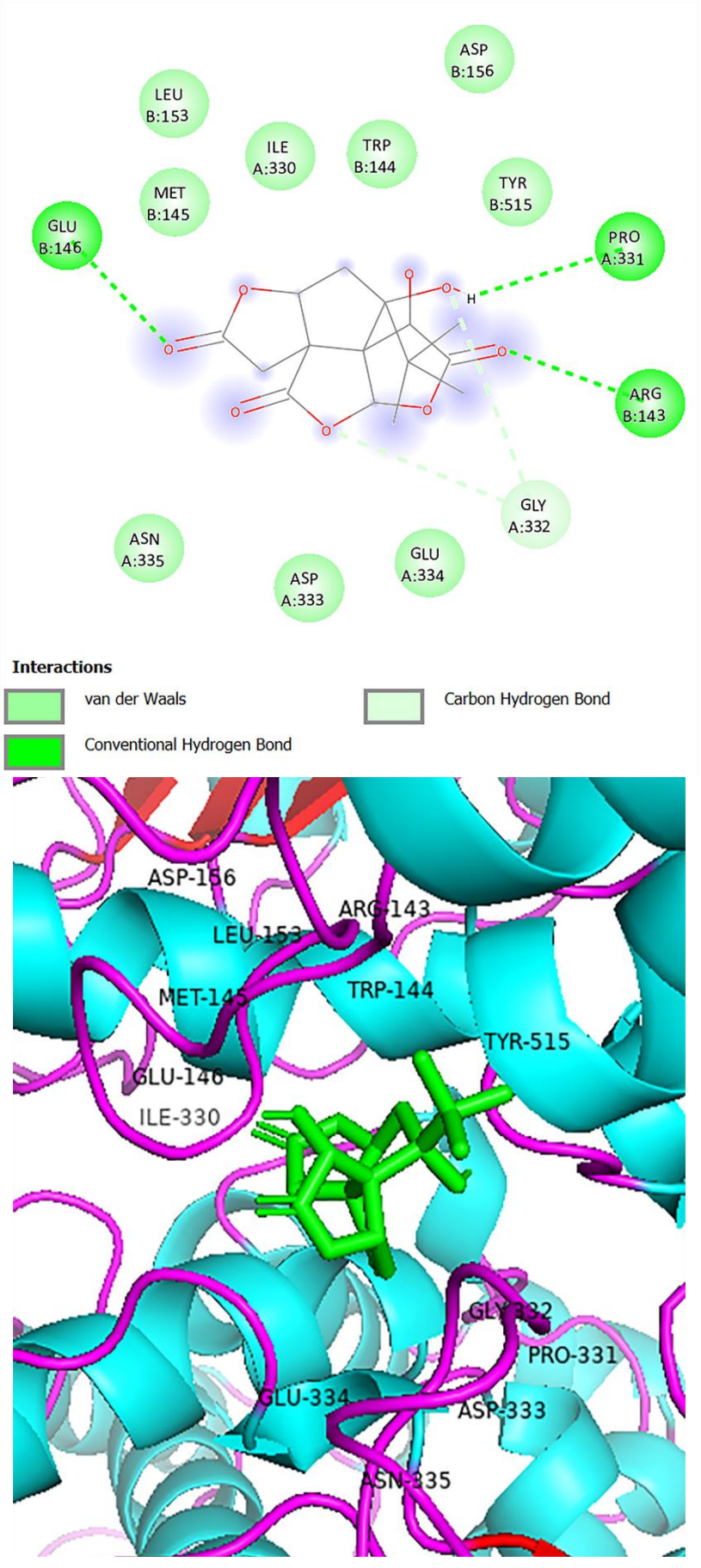
Ginkgetin


LYS B:409
ALA B:410
ILE B:406
GLN B:413
LEU B:414
ILE B:673
LEU B:368
VAL B:604
HIS B:600
LEU B:607
HIS B:372
GLN B:363
ALA B:603
PRO B:569
PHE B:359
HIS B:360
TRP B:599
ARG B:596
HIS B:432
Interactions
van der Waals
Conventional Hydrogen Bond
Pi-Donor Hydrogen Bond
Pi-Sigma
Pi-Pi T-shaped
Amide-Pi Stacked
Pi-Alkyl
ILE-673
LEU-607
HIS-372
VAL-604
ILE-406
GLN-363
LEU-368
ALA-603
ALA-410
LYS-409
HIS-600
HIS-360
TRP-599
HIS-432
GLN-413
ARG-596
LEU-414

Quercetin


TYR B:470
ILE B:454
ARG B:457
PHE B:450
VAL B:243
ASP B:442
PHE B:544
LEU B:448
SER B:447
GLN B:549
ALA B:453
ARG B:370
THR B:545
LEU B:244
VAL B:361
LEU B:288
THR B:371
THR B:366
ILE B:365
Interactions
van der Waals
Pi-Cation
Conventional Hydrogen Bond
Pi-Alkyl
PHE-450
SER-447
ASP-442
THR-371
ILE-454
ARG-370
ARG-457
TYR-470
THR-366
VAL-361

Bilobalide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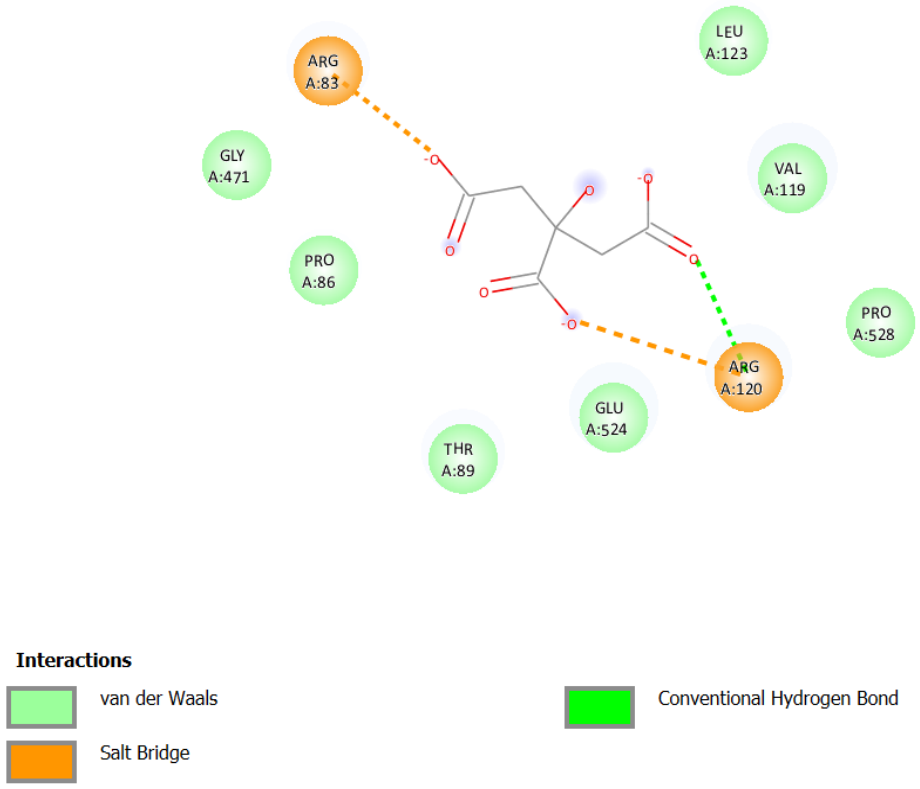
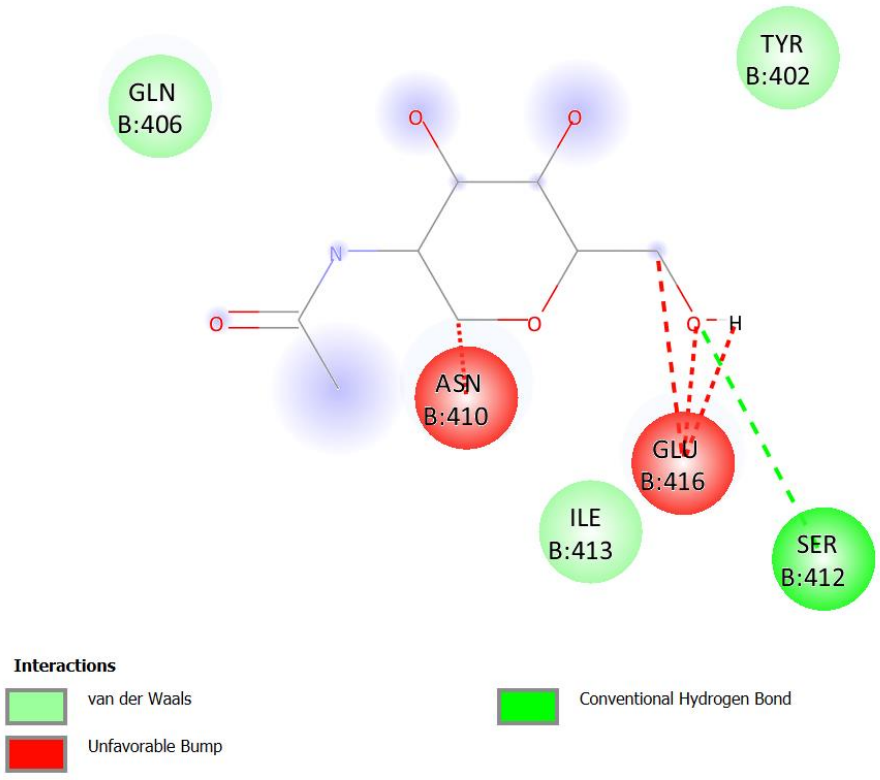
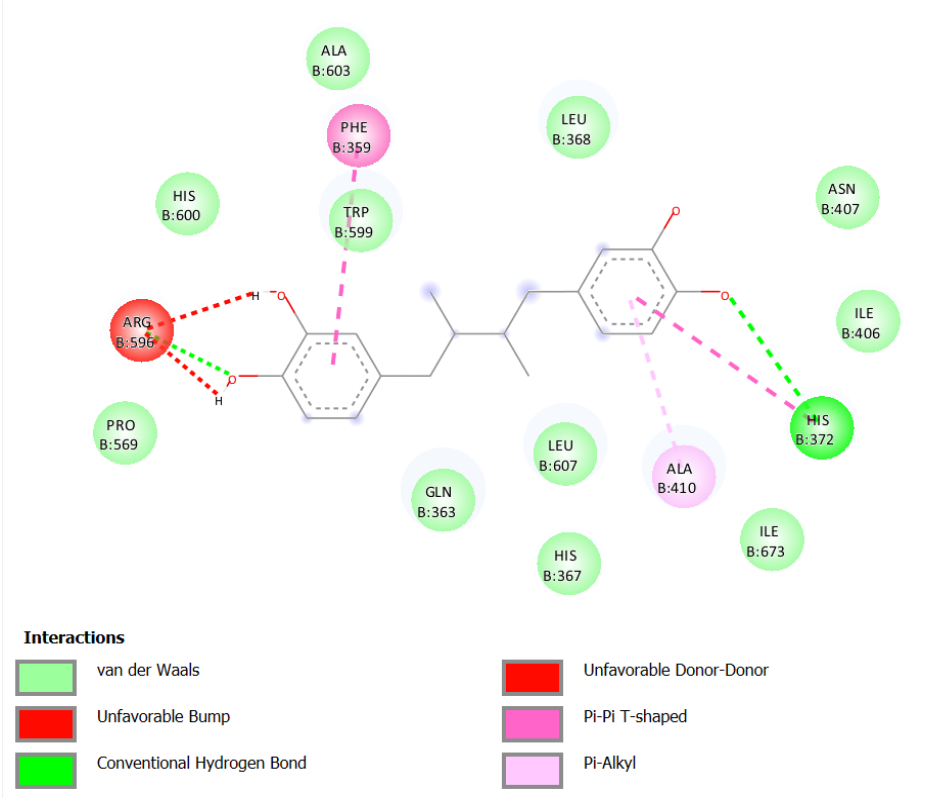
LEU B:153
ILE A:330
TRP B:144
TYR B:515
MET B:145
GLU B:146
PRO A:331
H
ARG B:143
GLY A:332
ASN A:335
GLU A:334
ASP A:333
Interactions
van der Waals
Carbon Hydrogen Bond
Conventional Hydrogen Bond
ASP-156
ARG-143
LEU-153
MET-145
TRP-144
TYR-515
GLU-146
ILE-330
GLY-332
PRO-331
GLU-334
ASP-333
ASN-335

Appendix 3. Receptor-ligand interactions visualization of protein and native ligands generated from re-docking

Protein	Ligand	Visualization
COX-1	FLC	
COX-2	NAG	

OX	30Z


ALA B:603
PHE B:359
LEU B:368
HIS B:600
TRP B:599
ASN B:407
ARG B:596
ILE B:406
PRO B:569
HIS B:372
LEU B:607
ALA B:410
GLN B:363
ILE B:673
HIS B:367
Interactions
van der Waals
Unfavorable Donor-Donor
Unfavorable Bump
Pi-Pi T-shaped
Conventional Hydrogen Bond
Pi-Alkyl