Chapter 5

# Molecular Docking Analysis: Interaction Studies of Natural Compounds to Anti-inflammatory Targets

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Additional information is available at the end of the chapter

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**Abstract**

A variety of compounds from medicinal plants have been reported to possess anti-inflammatory properties. Selected natural compounds that exhibit anti-inflammatory properties were subjected to docking simulation using AutoDock Vina to investigate their interaction modes to the potential macromolecular targets. The docking was performed using different molecular targets, i.e., cyclooxygenase-2, phospholipase A2, NF-κB inhibitor, and interleukin-1 receptor. It revealed that flavonoids have the highest affinity to the macromolecular targets (the lowest binding energy values) and the highest consistency of interaction model. Some terpenoids were identified to have potential inhibitor of phospholipase A2.

**Keywords:** molecular docking analysis, natural compounds, anti-inflammatory, Autodock Vina

## 1. Introduction

Inflammation is the body defense system in response to the pathogens and injury. During the inflammation process, various inflammatory mediators are synthesized and secreted from cells and generate many cellular effects [1]. Uncontrolled inflammation lead to several chronic diseases such as cardiovascular disease, arthritis, asthma, type 2 diabetes mellitus, and cancer. To relieve inflammatory responses, nonsteroidal anti-inflammatory drugs (NSAIDs) and steroids are widely used. NSAIDs possess anti-inflammatory effect by inhibit cyclooxygenase (COX) enzyme. However, their long-term use causes gastrointestinal toxicity due to nonselective inhibition of COX-1 and COX-2 [2]. Glucocorticoids decrease the transcription of proinflammatory cytokines and chemokines and increase the transcription of anti-inflammatory cytokines,

resulting in strong anti-inflammatory activity. However, their benefits are limited by the variety of systemic side effects and the development of resistance after chronic use. Thus, developing new drug candidates from natural products is greatly interesting [3].

In medicinal plants, many natural compounds, such as flavonoids, terpenoids, alkaloids, and saponin, have been reported to have in vitro as well as in vivo anti-inflammatory activity [4]. The mechanism of action and molecular target of various natural compounds needs to be studied for constructing a structure activity relationship. Molecular docking analysis can be conducted to study the interaction of these natural compounds with various molecular targets of anti-inflammatory activity. Further, the structure-activity relationship can be used to develop new derivative natural compounds with higher anti-inflammatory activity. This research aims to determine the model of interactions between the natural compounds with anti-inflammatory molecular target by molecular docking analysis. These natural compounds were used as the subjects in this study, with the cyclooxygenase-2 (COX-2), phospholipase A2, NF-κB–inducing kinase (NIK), and interleukin receptor (IRAK) as the molecular targets. The molecular docking analysis was conducted using Autodock Vina.

## 2. Molecular targets of anti-inflammatory agents

### 2.1. Cyclooxygenase-2 (COX-2)

The COX enzymes (COX-1 and COX-2) catalyze the biosynthesis of prostaglandins, prostacylins and thromboxanes, from arachidonic acid. COX-1 is constitutively expressed in most tissues, while COX-2 is expressed in specific tissues and is induced by cytokine and growth hormones. COX-1 possesses regulatory effects on platelet aggregation and gastric mucous biosynthesis, while COX-2 is involved in pathological conditions such as inflammation, pain, and fever. NSAIDs possess their anti-inflammatory activity by inhibition of COX-1 and COX-2. Prolonged inhibition of COX-1 in the gastrointestinal system causes gastrointestinal tract injury due to ulcer formation and gastric bleeding. Coxibs, the COX-2 selective inhibitor, were designed to inhibit COX-2 over COX-1, to obtain desired anti-inflammatory activity with minimal gastric toxicity side effect [5].

COX-1 and COX-2 were almost identical, despite of the residues of Ile434, His513, and Ile523 in COX-1, while in COX-2 were Val434, Arg513, and Val523. These differences result in a volume increasing of the COX-2 active site and additional side pocket off the main channel. The structures of coxibs consist of diaryl heterocycle with a sulfonamide or methyl sulfone moiety, which will bind to the side pocket of COX-2 to provide isoform-selective inhibition [6].

### 2.2. Phospholipase A2 (PLA2)

Phopholipase A2 (PLA2) enzymes are required to increase the level of arachidonic acid for metabolism and biosynthesis of eicosanoid under physiological condition as well as in inflammatory cell activation. PLA2 superfamily consists of cytosolic calcium dependent PLA2 (cPLA2), cytosolic calcium-independent PLA2 (iPLA2), and secreted PLA2 (sPLA2). iPLA2 is

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constitutively generating a low level of free fatty acids with relatively minimal specificity for the particular esterified fatty acid. cPLA2 hydrolysis arachidonic acid-containing phospholipids, leading to the production of proinflammatory eicosanoids. sPLA2 is an inducible enzyme that augments cPLA2 function to control the magnitude and duration of elevated free fatty acid levels including arachidonic acid [7].

### 2.3. NF-κB–inducing kinase (NIK)

Nuclear factor (NF)-κB is a group of eukaryotic transcription factors that regulates the expression of gene important for immune responses. NF-κB-inducing kinase (NIK) activates NF-κB2 by promoting proteolytic processing and the generation of NF-κB transcription of the targeted gene. NIK is also required in the signaling pathways elicited by other cytokines. NIK regulates both inflammation-induced and tumor-associated angiogenesis. NIK is highly expressed in endothelial cells of tumor tissues and inflamed rheumatoid arthritis synovial tissues [8].

### 2.4. Interleukin-1 receptor-associated kinase-4 (IRAK-4)

Contribution of interleukin-1 (IL-1), a proinflammatory cytokine, in the inflammation network is important. It propagates and amplifies signals; furthermore, the signaling pathways mediated by IL-1 and other cytokines receptors may communicate in various cross-talk mechanisms. Therefore, inhibition of IL-1 receptor would have profound effects on overall inflammatory responses. Interleukin-1 receptor-associated kinase 4 (IRAK-4) plays a pivotal role in signaling cascades associated with the immune and inflammatory diseases, and may be an effective therapeutic target for various diseases associated with deregulated inflammation [9].

## 3. Anti-inflammatory activity of natural compounds

### 3.1. Flavonoid and phenolic compounds

Flavonoids belong to a group of natural compounds and occur as aglycone, glycosides, and other derivatives. The flavonoids are categorized into flavonols, flavones, cathecins, flavanones, anthocyanidins, and isoflavonoids. Flavonoids exert their anti-inflammatory activity by various mechanisms, i.e., inhibition of phospholipase A2, COX, and LOX. Other mechanisms include inhibition of histamine release, phosphodiesterase, protein kinases, and activation of transcriptase. Phenylated flavonoids and a number of biflavonoids (amentoflavone, bilobetin, morelloflavone and ginkgetin) have been shown to inhibit phospholipase C1 and A2. Quercetin is reported as a strong inhibitor of both COX-2 and 5-LOX [10].

Curcumin, a polyphenol compound derived from the rhizomes of the plant turmeric, has anti-inflammatory activity, mainly due to inhibition of arachidonic acid (AA) metabolism, cyclooxygenase (COX), lipoxygenase (LOX), cytokines interleukin (IL) and tumor necrosis factor (TNF), and nuclear factor kappa B (NF-κB), despite it is also reported to stabilize lysosomal membranes [11–14].

### 3.2. Terpenoids

Terpenoids are classified into hemi-, mono-, sesqui-, di-, sester-, tri- and tetraterpenoids. A large numbers of terpenoids have been tested for anti-inflammatory properties. Anti-inflammatory activity of 1,8-Cineol, a monoterpene oxide, is correlated to inhibition of leukotriene B4, prostaglandin $E_2$ TNF-$\alpha$, interleukin, and thromboxane [15]. Parthenolide, a sesquiterpene lactone, possesses anti-inflammatory activity by several mechanisms, including inhibition of NF-κB [16]. Cucurbitacins, a group of triterpenes, were reported to show anti-inflammatory activity by inhibition of prostaglandin production and blocking NF-κB activation [17].

### 3.3. Alkaloid

Alkaloids are the basic substances that contain one or more nitrogen atoms, usually in combination as part of a cyclic system. They are often toxic to have various pharmacological activities, including anti-inflammatory activity [18]. Isoquinoline, quinoline, and indole alkaloids were the most studied classes for anti-inflammatory activity. Berberine, an isoquinoline alkaloid, showed potential in vitro and in vivo anti-inflammatory activity. It was reported to inhibit prostaglandin E2 production, without inhibitory effect on either COX-1 or COX-2 activity [19].

### 3.4. Saponins

Saponins are group of glycosides found in many plants, with triterpenoid or steroid as the aglycone moiety. Kalopanaxsaponin A was triterpenoid saponin isolated from the stem bark of *Kalopanax pictus* that showed significant anti-inflammatory activity [20]. Loniceroside A, a triterpenoid saponin isolated from the aerial parts of *Lonicera japonica*, also showed comparable anti-inflammatory activity to aspirin. It exhibited anti-inflammatory activity against acute and chronic inflammation [21].

## 4. Molecular docking analysis

Molecular modeling investigations were carried out using Zyrex Cruiser workstation EM4100 running Intel Core i3-4030U Processor, 2 GB RAM, 500 GB hard disk, and Intel HD Graphic Family graphics card. Autodock Vina docking program, Molecular Graphic Lab, The Scripps Research Institute [22] was employed for the docking studies.

### 4.1. Preparation of target proteins

PDB structures used, i.e., 5IKT (COX-2), 4UY1 (PLA2), 1DV4 (NF-κB), 5KX7 (IRAK-4), were obtained from the Brookhaven Protein Data Bank (www.rcsb.org). **Table 1** presented the

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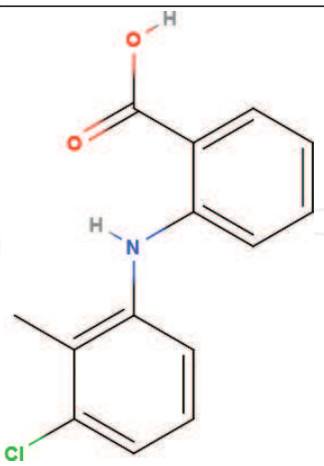
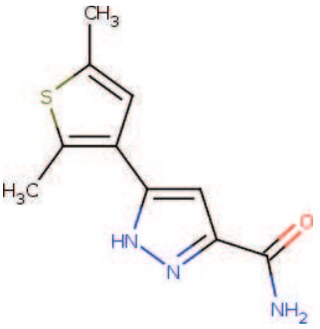
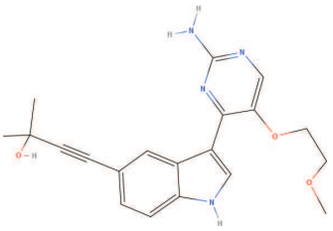
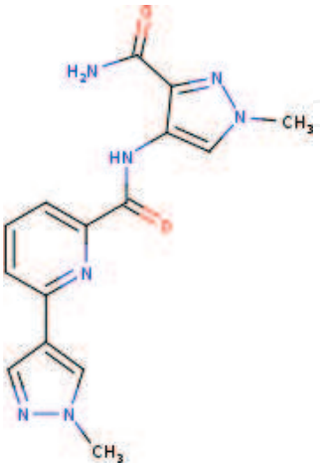
| Target macromolecule (pdb code) | Ligand | Structure |
|---|---|---|
| COX-2 (5IKT) | Tolfenamic acid |  |
| Phospholipase A2 (4UY1) | 5-(2,5-dimethyl-3-thienyl)-1H-pyrazole-3-carboxamide |  |
| NIK (IDV4) | 4-{3-[2-amino-5-(2-methoxyethoxy)pyrimidin-4-yl]-1H-indol-5-yl}-2-methylbut-3-yn-2-ol |  |
| IRAK-4 (5KX7) | ~{N}-(3-aminocarbonyl-1-methyl-pyrazol-4-yl)-6-(1-methylpyrazol-4-yl) pyridine-2-carboxamide |  |

**Table 1.** Target macromolecules, pdb codes, native ligands, and ligand structures used in the docking study.

target macromolecules and each of the native ligand. The protein structures were prepared using UCSF Chimera 1.11.2 to remove all nonreceptor atom including water, ion, and miscellaneous compounds. The obtained structures then were saved as pdb file.

### 4.2. Preparation of ligands

The structures of native ligands from each target macromolecules were prepared by UCSF Chimera 1.11.2 to separate from the protein, water, and miscellaneous substances. The structures of the 40 ligands of natural compounds were sketched using MolView 2.2. Each structure then was executed an MMFF94 energy minimization. These obtained conformations were used as starting conformations to perform docking analysis.

### 4.3. Docking method validation

To ensure that the docking studies were valid and represented the reasonable potential binding model, the docking methods and parameters used were validated by redocking experiment. Each copy of native ligand was docking into the native protein to determine the ability of Autodock program to reproduce the orientation and position of the ligand observed in the crystal structure. The valid criteria used is the all atom root mean square deviation (RMSD) between the docked position and the crystallographically observed binding position of the ligand, and success is typically regarded as being less than 2 Å.

### 4.4. Docking studies

Docking studies were carried out using the above mentioned prepared target macromolecules and natural compound ligands (1–40) by employing Autodock Vina program. Docking was performed to obtain a population of possible conformations and orientations for the ligand at the binding site. The protein was loaded in PyRx software, creating a PDBQT file that contains a protein structure with hydrogens in all polar residues. All bonds of ligands were set as rotatable. All calculations for protein-fixed ligand-flexible docking were done using the Lamarckian Genetic algorithm (LGA) method. The docking site on protein target was defined by establishing a grid box with a default grid spacing, centered on the position of native ligand. The best conformation was chosen with the lowest binding energy, after the docking search completed. The interactions complex protein-ligand conformations, including hydrogen bonds and the bond lengths were analyzed using Discovery Studio Visualizer 16.1.0.15350.

## 5. Result and discussion

The molecular docking analysis was performed using Autodock Vina program with 40 plant-derived compounds, including flavonoids and phenolic compounds, alkaloid, terpenoid, and saponin. First, the validation method was conducted to ensure the capability of docking machine. **Figure 1** represented the validation result of docking protocol. All of the redocking ligands showed similar conformation with the native redocking ligands, and the RMSD values were ≤2.0 Å.

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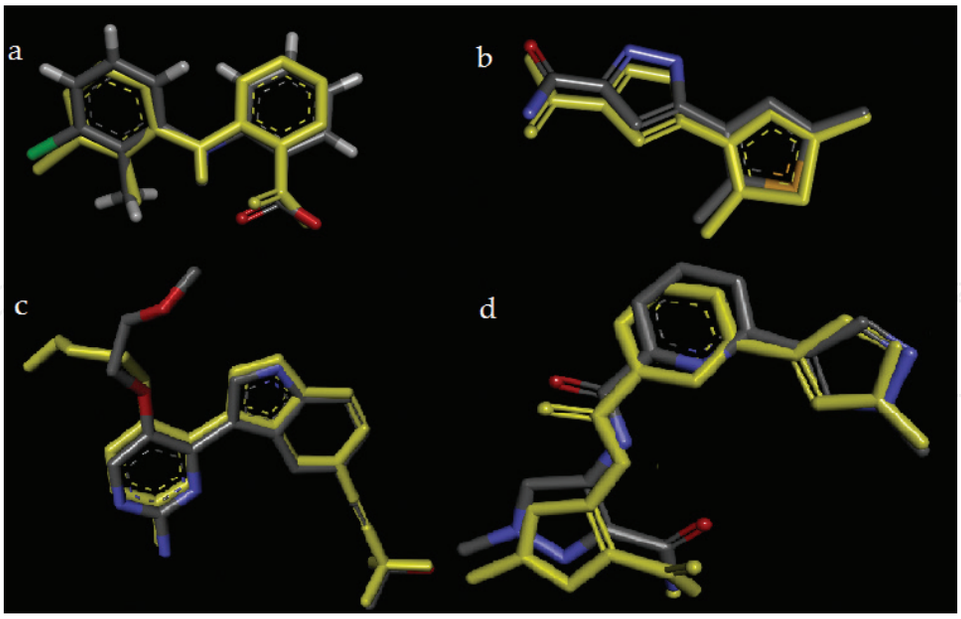


**Figure 1.** Overlay of the native ligands (gray) and redocking conformations (yellow). (a: COX-2; b: PLA2; c: NF-κB; d: IRAK-4).

Each natural compound then was docked into each of six different targets, using valid parameter method. The lowest energy docked conformation of the best cluster was selected and analyzed. **Table 2** summarizes the docking study results, presented as binding energy.

### 5.1. Interaction to Cyclooxygenase-2

Structure of COX-2 complex with tolfenamic acid, a selective COX-2 inhibitor, was used for this study. The carboxylic group of tolfenamic acid interacted by hydrogen bonding with Tyr-385A and Ser-530A at the top of channel. While the methylphenyl aminobenzoic moiety interacted hydrophobically with Val116A, Val349A, Leu352A, Val523A, Ala527A, as well as Leu531A [23]. This study revealed that some flavonoids and phenolic compounds, i.e., amentoflavone, apigenin, bilobetin, diosmine, epicatechin gallate, ginkgetin, hesperidin, luteolin, morelloflavon, and quercetin, showed lower binding energy than that of tolfenamic acid, the selective COX-2 inhibitor.

The model interaction of these flavonoids to the binding site of COX-2 was similar to the model interaction of native ligand. Carbonyl group at C-ring of flavonoid played an important role in the ligand-target interaction, by hydrogen bond interaction to Ser530A and Arg120A residue, while A- and B-phenolic ring interacted to Val349A, Leu352, Ala527, and Leu531A residue via hydrophobic interaction (**Figure 2**).

This result was in line with previous reports about COX-2 inhibitory activity of flavonoid and phenolic compounds. Several research reports about COX inhibitory activity of flavonoids were documented [24–26]. A structure-activity relationship study about COX-2 inhibitory activity of flavonoid resumed the pharmacophores group were 4-oxo (C-ring), 7-hydroxyl moiety (A-ring), as well as para-substituted phenolic B-ring [27]. Apigenin meets all the requirements, due to its hydroxylation pattern at 5′-, 7′-, and 4′-positions.

| Compound | Target macromolecules; binding energy (kcal/mole) | | | |
|---|---|---|---|---|
| | **COX-2** | **PLA2** | **NIK** | **IRAK-4** |
| Native ligand | −9.0 | −7.8 | −9.9 | −9.0 |
| **Flavonoids and phenolic compounds:** | | | | |
| Amentoflavone | −8.7 | −9.0 | −8.2 | −8.1 |
| Anthocyanin | −8.3 | −7.8 | −8.3 | −8.1 |
| Apigenin | −9.2 | −7.8 | −9.1 | −9.3 |
| Apocynin | −6.7 | −6.5 | −6.8 | −6.3 |
| Apocynin ester | −6.2 | −5.8 | −6.0 | −6.2 |
| Bilobetin | −10.6 | −9.1 | −6.3 | −10.1 |
| Curcumin | −8.9 | −8.4 | −9.0 | −9.3 |
| Diosmine | −8.6 | −8.9 | −5.6 | −8.8 |
| Epicatechin gallate | −9.9 | 8.2 | −7.9 | −8.4 |
| Epigallocatechin gallate | −8.4 | 8.1 | −8.0 | −8.8 |
| Ginkgetin | −9.4 | −9.0 | −6.4 | −10.2 |
| Hesperidin | −8.8 | −8.9 | −6.1 | −8.2 |
| Hydrocinnamic acid | −6.4 | −6.6 | −6.4 | −6.8 |
| Luteonin | −9.4 | −8.1 | −9.3 | −8.9 |
| Morelloflavon | −9.5 | −8.1 | −4.1 | 3.0 |
| Paeonol | −6.5 | −6.0 | −5.9 | −6.1 |
| Procyanidin | −7.9 | −8.5 | −6.5 | −4.9 |
| Pterostilben | −7.6 | −6.7 | −8.0 | −8.0 |
| Quercetine | −9.5 | −8.1 | −8.9 | −9.1 |
| Resveratol | −7.9 | −7.1 | −8.0 | −8.1 |
| Yuccaol A | −7.8 | −8.2 | −6.4 | 0.3 |
| Yuccaol B | −8.0 | −7.0 | 0.3 | −0.8 |
| Yuccaol C | −7.8 | −6.9 | −0.4 | −0.5 |
| Yuccaol D | −8.5 | −7.5 | −1.8 | 3.3 |
| Yuccaol E | −8.1 | −7.1 | −0.9 | −1.0 |
| **Alkaloids** | | | | |
| Berberine | −9.8 | −8.4 | −8.9 | −9.3 |
| **Terpenoids** | | | | |
| 1.8-Cineol | −5.6 | −5.2 | −5.8 | −5.9 |
| Cucurbitacin B | −8.0 | −7.6 | −2.1 | −0.6 |

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| Compound | Target macromolecules; binding energy (kcal/mole) | | | |
|---|---|---|---|---|
| | COX-2 | PLA2 | NIK | IRAK-4 |
| Cucurbitacin D | −8.2 | −7.4 | −3.3 | −1.7 |
| Cucurbitacin E | −8.8 | −7.8 | −2.9 | −3.3 |
| Cucurbitacin I | −8.4 | −7.3 | −3.7 | −3.0 |
| Cucurbitacin R | −8.3 | −7.9 | −2.6 | −1.3 |
| Dihydrocucurbitacin B | −7.5 | −7.2 | −2.1 | −1.8 |
| Oleanolic acid | −8.7 | −7.5 | 1.3 | −3.0 |
| Partenolid | −7.7 | −8.1 | −6.7 | −7.3 |
| Pseudopterosin | −7.8 | −7.5 | −8.3 | −9.0 |
| Ursolic acid | −7.8 | −8.0 | 1.7 | −3.6 |
| **Saponins** | | | | |
| Kalopanaxsaponin | −8.7 | −7.7 | 9.1 | −0.5 |
| Loniseroside A | −6.1 | −7.8 | −7.4 | −6.5 |

**Table 2.** Binding energy values of natural compounds docked to target macromolecules.

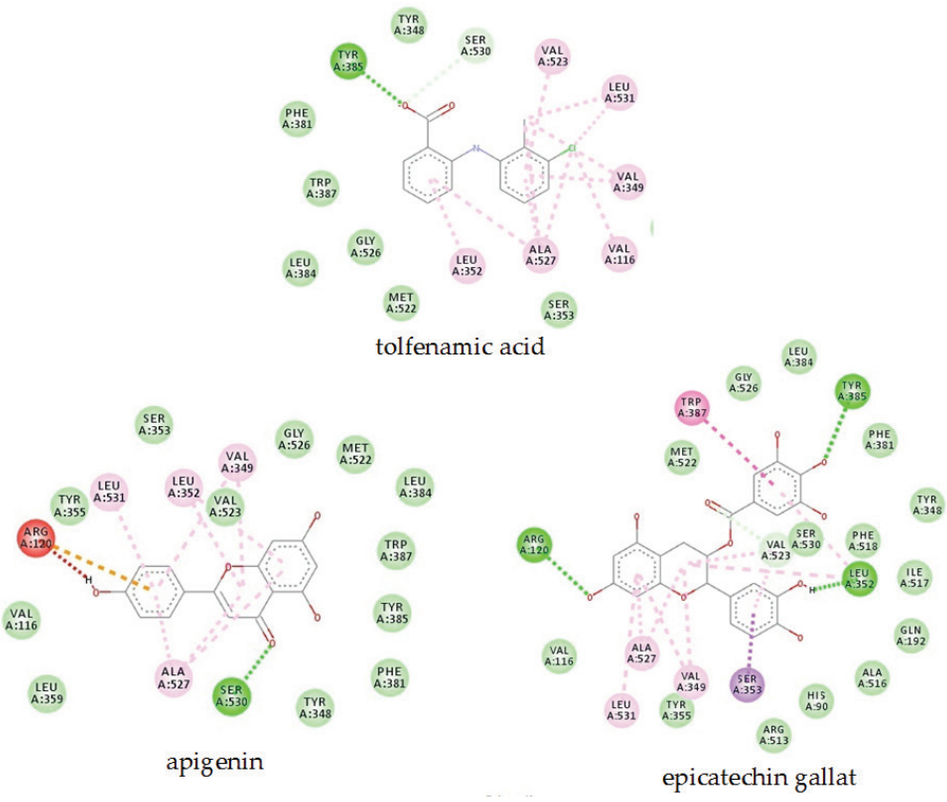


**Figure 2.** Model interaction of tolfenamic acid, apigenin and epicatechin gallate with COX-2 binding site. Dark green: hydrogen bond; light green: van der Waals; red: electron donor-donor interaction; pink-violet: hydrophobic interaction.

Epicatechin gallate was reported to inhibit COX-2 enzyme [28]. In this study, epicatechin gallate also showed good interaction to COX-2 binding site, where hydrogen bond was formed between its phenolic groups and Tyr385A as well as Arg180A residues, in addition to hydrophobic interactions between the aromatic groups and Val349A, Val523A, Ala527A, and Leu531A. Berberine, the alkaloid used in study, showed lower affinity compared to native ligand. This result was in agreement with the fact that berberine does not interfere with COX enzyme activity [19].

### 5.2. Interaction to Phospholipase A2

Complex structure of PLA with 5-(2,5-dimethyl-3-thienyl)-1H-pyrazole-3-carboxamide, shows that the terminal amide group interacts with by hydrogen bonding with Asp47A and His46A, while hydrophobic interaction is formed between the thienyl pyrazole moiety and Leu5A [26]. Three biflavonoids, i.e., amentoflavone, bilobetin, and ginkgetin, were the ligands with the lowest binding energy, with the binding energy value being −9.0, −9.1, and −9.0 kcal/mole respectively. Biflavanoids were repeatedly reported as PLA2 inhibitors [29–32].

The biflavonoids successfully docked into the PLA2 binding site (**Figure 3**). The compounds bind to the target via hydrogen bond with Leu29A residue, while hydrophobic interactions were formed between aromatic rings of biflavonoid with Ala2A, Leu5A as well as Ala6A. Hydrophobic pharmacophores of biflavonoids support the PLA2 inhibitory activity, due to interaction between substrate of PLA2 usually occurs at hydrophobic channel of the enzyme.

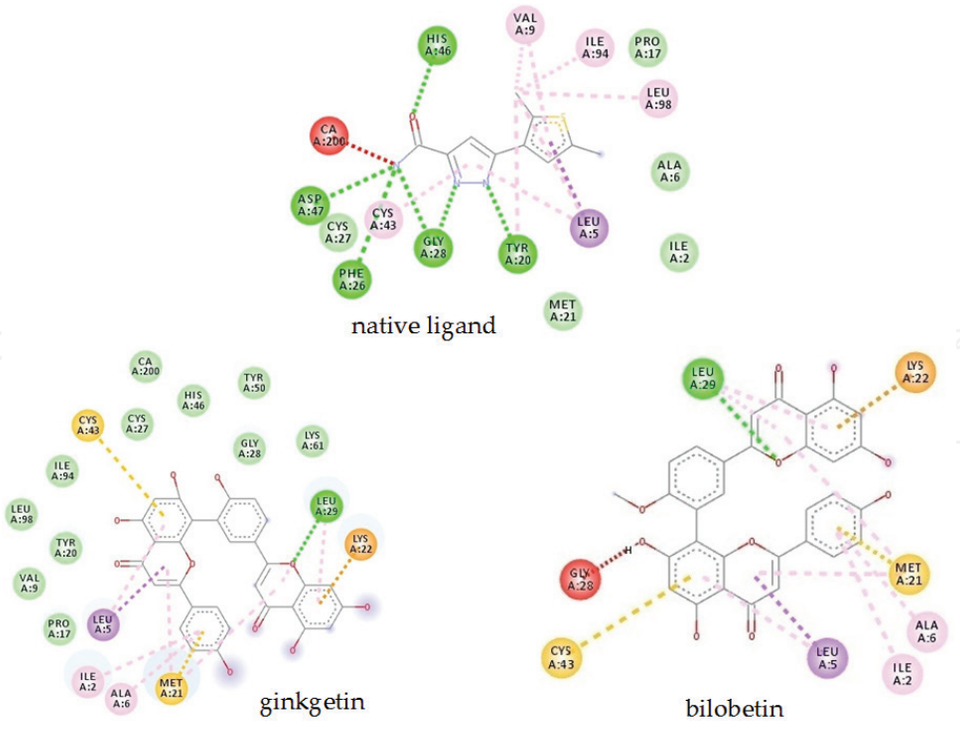


**Figure 3.** Model interaction of phospholipase native ligand, bilobetin, and ginkgetin. Dark green: hydrogen bond; light green: van der Waals; red: electron donor-donor interaction; pink-violet: hydrophobic interaction; brown: π-cation interaction.

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The π-cation interaction increases the affinity of biflavonoid, because the $Ca^{2+}$ ion is important for the catalytic mechanism of PLA2 [33].

### 5.3. Interaction to NF-κB–inducing kinase (NIK)

Study about NIK inhibitory has been conducted with the analogs of imidazopyridinyl pyrimidinamine. The hydrogen bonds were formed between pyrimidinamine group and Glu470A as well as Leu 472A; furthermore, imidazopyridinyl pyrimidinamine ring was hydrophobically interacted to Val414A, Ala427A, Lys429A, Leu522A, and Cys533A. Interaction of π-sulfur was formed between pyrimidinamine ring and Met469A [34].

This study revealed that apigenin and luteolin, two flavonoid aglikons, had lowest binding energy values. This was in line with previous research reports that apigenin and luteolin inhibit activation of NF-πB [35–38].

The similarity of interaction model between native ligand and these flavonoids are presented in **Figure 4**. Hydrogen bond was formed between carbonyl group at C-ring (4-oxo) of flavonoid and Ser476A residue. Similar to the interaction model of native ligand, hydrophobic interactions were formed between aromatic rings of flavonoid with Val414A, Ala427A, and Leu522A residues. Interaction of π-sulfur was also formed between B-ring with Met469A residue. Additional hydrogen bonding was formed between hydroxyl groups of B-ring with Glu470A and Leu472A residues. This result concluded that *o*-catechol or *p*-hydroxy B-ring

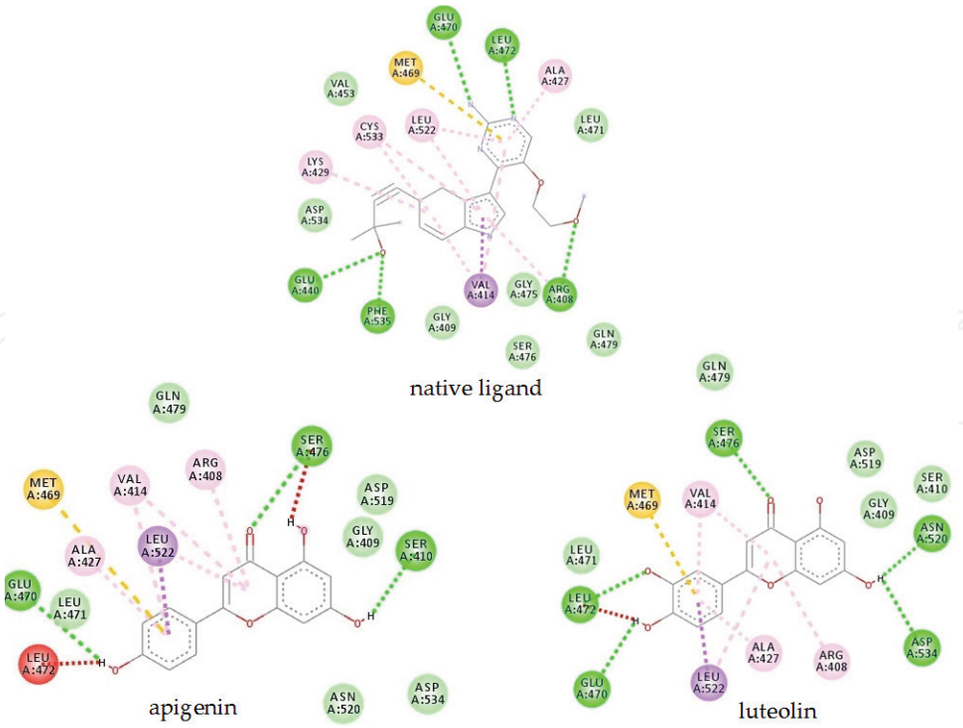


**Figure 4.** Model interaction of NIK native ligand, apigenin, and luteolin. Dark green: hydrogen bond; light green: van der Waals; red: electron donor-donor interaction; pink-violet: hydrophobic interaction; brown: π-sulfur interaction.

was the pharmacophore group for NIK inhibitory activity, in addition to -oxo and planar aromatic ring of flavonoids.

### 5.4. Interaction to Interleukin-1 receptor-associated kinase-4 (IRAK-4)

The X-ray crystal structure of IRAK-4-inhibitor complex has been reported. The structure revealed that the carboxamide group and N-pyrazol contributed as hydrogen bond acceptor (interacted to Met265B and Lys213B, respectively). Furthermore, two aromatic rings acted as hydrogen bond donor increased the affinity to the target via hydrophobic interaction with Val200B, Ala211B, Val246B, Tyr262B, as well as Leu318B [39].

Among 40 natural compounds docked to IRAK-4, apigenin, bilobetin, curcumin, ginkgetin, quercetin, berberine, and pseudopterosin showed higher affinity compared to the native ligand. Quercetin was reported to show IRAK-4 inhibitory activity [40]. Affinity of quercetin to the binding site was supported by hydrogen bond between 4-hydroxy of B-ring with Lys213B and Glu233B residues, while the aromatic rings contributed by forming hydrophobic interaction with Val200B, Tyr262B, and Leu318B. Similar to the interaction model of native ligand to IRAK-4, aromatic rings of bilobetin also formed the hydrophobic interaction to Val200B, Tyr262B, and Leu318B residues of IRAK-4 (**Figure 5**).

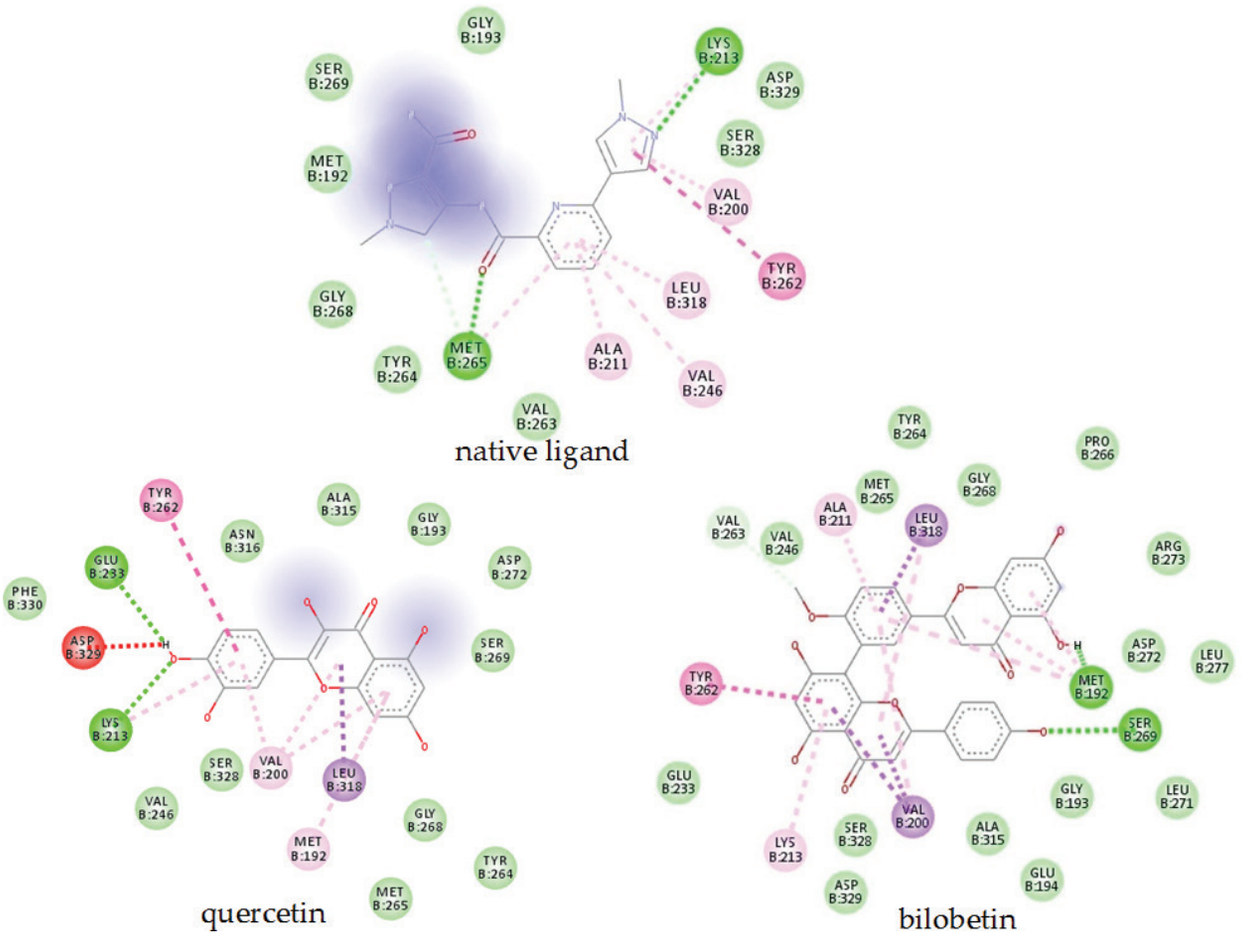


**Figure 5.** Model interaction of NIK native ligand, quercetin, and bilobetin. Dark green: hydrogen bond; light green: van der Waals; red: electron donor-donor interaction; pink-violet: hydrophobic interaction.

## 6. Conclusion

The docking results revealed that the highest affinity to the macromolecular targets (the lowest binding energy values) and the consistency of interaction model were shown by the flavonoids. Some terpenoids were identified to have potential inhibitor of phospholipase A2.

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