Vietnam Journal of Science and Technology ***60*** *(5) (2022) 785-793*

doi:10.15625/2525-2518/16469

EVALUATION OF ANTI-INFLAMMATORY COMPOUNDS ISOLATED FROM *MILLETTIA DIELSIANA* HARMS EX DIELS BY MOLECULAR DOCKING METHOD

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Received: 23 August 2021; Accepted for publication: 27 July 2022

Abstract. In this study, we focused on screening and simulating the interaction between anti-inflammatory proteins and 50 compounds isolated from *Millettia dielsiana* Harms ex Diels. 39 out of 50 compounds that violated no of Lipinski's rule of five were sorted out as favorable for drug development and selected for studies further. Then, a molecular docking study of compounds into the binding sites of COX-1 and COX-2 allowed shedding light on the binding mode of these potential COX inhibitors performed using Autodock Vina software. Our results showed that 6 compounds, including millesianin E (**D32**), barbigerone (**D18**), millesianin D (**D31**), (+)-epicatechin (**D11**), durallone (**D17**), and ichthynone (**D19**) exhibited good binding energy with the cyclooxygenase-2 (COX-2) enzyme meanwhile all of the selected compounds exhibited poor binding energy to the cyclooxygenase-1 (COX-1) enzyme. The binding energies of these compounds range from -8.6 kcal/mol to -9.0 kcal/mol better than the standard compounds Valdecoxib and Lumiracoxib. In addition, an analysis of the COX-2 enzyme and selected compounds binding was also presented. The important binding modes shown at the active site of the COX-2 enzyme through hydrogen bonds compared with standard compounds showed this as potential candidates against this enzyme. Therefore, these results might give a positive signal in finding anti-inflammatory drugs from *Millettia dielsiana*.

Keywords: molecular docking, cyclooxygenase-1, cyclooxygenase-2, *anti*-inflammation, *Millettia dielsiana*.

Classification numbers: 1.2.1, 1.2.4.

1. INTRODUCTION

Inflammation is a natural response of the immune system that protects the body against invaders. Several symptoms such as swelling, heat, redness and pain are usually included in the inflammation process because of the dilation of blood vessels which bring blood and white blood cells to the site of injury. The white blood cells produce agents to destroy or neutralize invaders. Prolonged inflammation may result in chronic inflammation and cause inflammatory diseases [1]. Mechanism of inflammation caused by an agent acting in the cell membrane leads to the activation of several regulatory enzymes such as phospholipase A2, lipoxygenase, and

cyclooxygenase that release arachidonic acid and inflammatory mediators such as cytokines, histamine, serotonin, leukotrienes, prostaglandins causing tissue cell damage and circulatory disorders [1, 2]. Non-steroidal anti-inflammatory drugs (NSAIDs) are commonly used in the treatment of inflammation. However, they inhibit both isomers of the cyclooxygenase enzymescyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2). Of these, the inhibition of COX-1 enzyme leads to damage of the gastrointestinal mucosa. Meanwhile, selective inhibition of COX-2 enzyme does not affect gastrointestinal tract but increases the ability to reduce inflammation during therapy [3]. Therefore, selective COX-2 inhibitors are potential anti-inflammatory agents. In recent years, with the development of Bioinformatics, potential enzymatic inhibitors can be predicted more easily and faster by simulating their binding reactions with corresponding pathogenic proteins.

Millettia dielsiana Harms ex Diels is a woody vine plant belonging to the Fabaceae family [4]. It grows in closed evergreen forests or sometimes in semi-dry, semi-deciduous forests and is mainly distributed in Laos, China, and Viet Nam [5, 6]. Previous reports indicate that flavonoids are major chemical components and responsible for the anti-inflammatory activity of this plant. These compounds inhibit the biosynthesis of nitric oxide (NO), which plays an important role in regulating inflammation and also reduces the risk of chronic inflammatory diseases [7 - 10]. Furthermore, flavonoids are involved in the mechanism of action of eicosanoid-producing enzymes (cyclooxygenases) [11]. The structure of some flavonoid compounds also influences their inhibitory capacity on COXs, with each enzyme being strongly or weakly inhibited depending on the substituted group position on the flavonoid compound's ring. Only the structure of the flavonoid compounds in the B ring that contained at least an OH group or have a double bond at the C2-C3 position of the C ring inhibited COX-1 most effectively. In contrast to COX-1, COX-2 was most inhibited when the flavonoid structure contained a catechol group in the B ring [12].

Therefore, in this study, we focused on *in silico* screening on the COX-1 and COX-2 inhibitory activities of selected compounds from the *M. dielsiana* by molecular docking method.

2. MATERIALS AND METHODS

2.1. Ligand selection and preparation

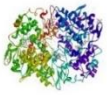
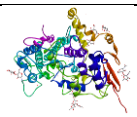
Isolated compounds from *M. dielsiana* were collected from previous studies [7 - 10] and described in Appendix 1. Their chemical structures were drawn from the Marvin software [13]. The three-dimensional structures as pdb files were built using Discovery Studio Visualizer software version 19.1.0.18287 (BIOVIA, San Diego, CA, USA) [14]. The geometric structures of these compounds were optimized by the Gabedit 2.5.0 software with hybrid B3LYP density functional theory using 6-311 + G(d,p) basis sets [15] and were then converted to pdbqt file using MGLTools v1.5.6 software [16].

2.2. Protein selection and preparation

The targeted COX-1 and COX-2 proteins were downloaded from the RCSB protein databank (https://www.rcsb.org/) with identifiers PDB IDs 6Y3C [17] and 5KIR [18], respectively. Information about the resolution, chains, co-crystallized ligand in the model, and X-ray crystallized structure are summarized in Table 1. The downloaded proteins were then

treated to remove water molecules, and original ligands, adding the polar hydrogens, and Gasteiger charges calculations [19].

Table 1. Some information about the protein model.

PDB ID	Resolution	Chains	Co-crystallized ligand	Crystallized three-dimensional structure
5KIR (COX-2)	2.70 Å	A, B	COH, RCX, GOL, NAG	
6Y3C (COX-1)	3.36 Å	A	FLC	

2.3. Lipinski's Rule

Based on Lipinski's rule, molecules with potent medicinal properties are selected according to several criteria such as molecular mass less than 500 Daltons, number of acceptor hydrogen bonds less than 10, number of donor hydrogen bonds less than 5 and LogP value less than 5 [20, 21]. The DruLito software [22] was selected to calculate the molecular weight and determine the above parameters.

2.4. Preparing parameters and performing molecular docking simulation using Autodock Vina

An Autodock software tool was applied to create grid boxes containing the active region of the compound in the binding simulation. The size of the grid boxes was set at 30×30×30 with the grid points spacing being 1 Å. The centre coordinates of the grid boxes are detailed in Table 2. The exhaustiveness setting was defined as 8 by default. The parameter files were prepared for molecular docking simulation using Autodock Vina version 1.1.2 [23]. Docking results were analyzed using Discovery Studio Visualizer software version 19.1.0.18287 (BIOVIA, San Diego, California, USA) [14].

Table 2. The parameters of the grid boxes are generated based on the compound positions included in the model of the target proteins.

Target protein	Original ligand	Coordinates at the center of the grid box		
		X	Y	Z
5KIR	RCX	23.21	1.32	34.26
6Y3C	FLC	-0.77	-59.35	6.41

3. RESULTS AND DISCUSSION

3.1. Compounds that obey Lipinski's rule

The Lipinski's rule is commonly used to assess the potentials between drugs and target non-pharmaceutical molecules. It acts as a filter to filter the active ingredients/medications for oral administration. In this way, it can reduce costs, time and effort, generate data sets related to

clinical drug development and to a large extent, reduce the failure of advanced clinical trials [20,21]. Among 50 compounds isolated from *M. dielsiana*, 11 compounds (**D7**, **D15**, **D38**, **D39**, **D41**, **D42**, **D45**, **D47**, **D48**, **D49**, and **D50)** violated Lipinski's rule. Particularly, three compounds (**D7**, **D41**, and **D47**) violated in 1 criterion. Seven compounds (**D15**, **D38**, **D39**, **D42**, **D45**, **D48**, and **D50**) violated in 2 criteria (Appendix 2). Other compounds that followed Lipinski's rule are promising with properties similar to oral medications and potential for further development into oral medications.

3.2. Molecular docking simulation results

Compounds that passed Lipinski's rule were used to continuously perform molecular docking simulation. Docking results of 39 compounds at in active regions of the 2 proteins were then compared with those of the positive controls. Aspirin and a member of the nonsteroidal anti-inflammatory drugs (NSAIDs), Naproxen, were selected to be positive controls. They are commonly used as anti-inflammatory agents, painkillers, and antipyretics by inhibiting the activity of the COXs [24]. Both of these positive drugs are not selective inhibitors either for COX-1 and COX-2, causing side effects with long-term use such as disorders, ulcers, stomach, and intestines [25]. Other selective inhibitors of COX-2 (Lumiracoxib, Valdecoxib) [26] and COX-1 (Ketoprofen, TFAP) [27] were used as additional positive drugs to clearly address that the screened compounds are selective inhibitors or non-selective inhibitors.

As shown in Table 3, both Naproxen and Aspirin gave a binding affinity ratio between COX-2 and COX-1 of approximately 1 unit/mol which are considered COXs non-selective inhibitors. And then, the binding affinity ratios ($\Delta G_1^{ref}/\Delta G_2^{ref}>1$) and ($\Delta G_1^{ref}/\Delta G_2^{ref}<1$) indicated COX-2 selective inhibitors and COX-1 selective inhibitors, respectively, as shown by positive drugs Valdecoxib, Lumiracoxib, Ketoprofen, and TFAP. These results are consistent with experimental data reported previously [26, 27]. Therefore, similar molecular docking parameters were chosen to investigate selected compounds from *M. dielsiana.*

Table 3. Binding affinity results of the positive drugs with COXs proteins.

No.	Ligand	ΔG_1^{ref}	ΔG_2^{ref}	$\Delta G_1^{ref}/\Delta G_2^{ref}$
COXs Non-selective inhibitors				
1	Naproxen	-8.0	-8.0	1.00
2	Aspirin	-7.1	-6.3	1.13
COX-2 selective inhibitors				
3	Valdecoxib	-8.8	-7.0	1.28
4	Lumiracoxib	-8.5	-6.3	1.35
COX-1 selective inhibitors				
5	Ketoprofen	-7.8	-8.8	0.89
6	TFAP	-7.3	-8.3	0.88

ΔG_1^{ref}: Binding affinity of the compound at the active site of the COX-2 enzyme (5KIR).

ΔG_2^{ref}: Binding affinity of the compound at the active site of the COX-1 enzyme (6Y3C).

After simulation, our results showed that in the active region of COX-2 enzyme (PDB ID 5KIR) there are 12 compounds with binding affinity ranging from -8.5 kcal/mol to -9.9 kcal/mol better than the binding affinity strength of the Lumiracoxib control and in COX-1 enzyme active region (PDB ID 6Y3C) there are 12 compounds with binding affinity ranging from -8.3 kcal/mol to -11.1 kcal/mol better than the affinity binding strength of the control TFAP (Appendix 3). In

addition, compounds **D22**, **D10**, **D12**, **D20**, **D21**, and **D36** are not considered because they are non-selective, they have a strong binding affinity to the two COX enzymes. This may lead to unwanted side effects. Potential anti-inflammatory compounds with a good binding affinity to the active region of the COX-2 enzyme and a low binding affinity to the COX-1 enzyme presented in Table 4 demonstrated high selectivity.

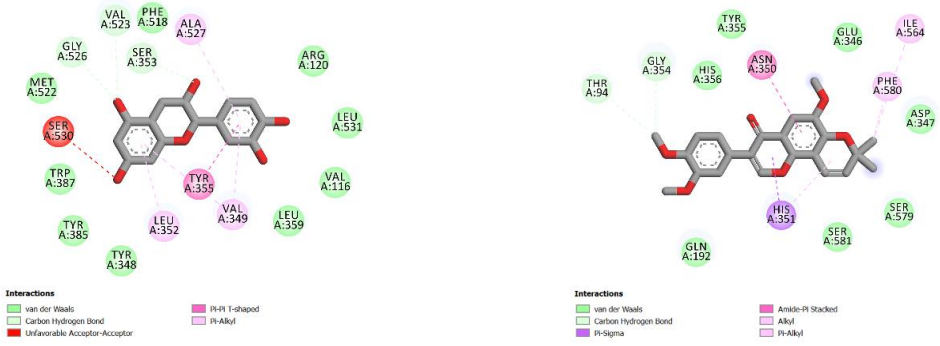
Table 4. Results of compounds with the best binding affinity and selectivity for COX-2 enzyme.

No.	Ligand	ΔG_1 (kcal/mol)	ΔG_2 (kcal/mol)	Hydrogen bonds	Number of hydrogen bond
1	D32	-9.0	-7.0	ASP347, PHE580, SER581, GLN192	4
2	D19	-8.9	-7.1	ARG120	1
3	D18	-8.6	-7.0	-	0
4	D31	-8.6	-6.8	ARG120	1
5	D11	-8.6	-7.2	-	0
6	D17	-8.6	-7.4	-	0
7	Valdecoxib	-8.8	-7.0	PHE518, LEU 352, SER 353, GLN 192, ARG 513	5
8	Lummiracoxib	-8.5	-6.3	-	0
9	Aspirin	-7.1	-6.3	ALA 527, MET 522	2
10	Naproxen	-8.0	-8.0	ARG 120	1

ΔG_1: Binding affinity of the investigated compound to the COX-2 enzyme (PDB ID: 5KIR).

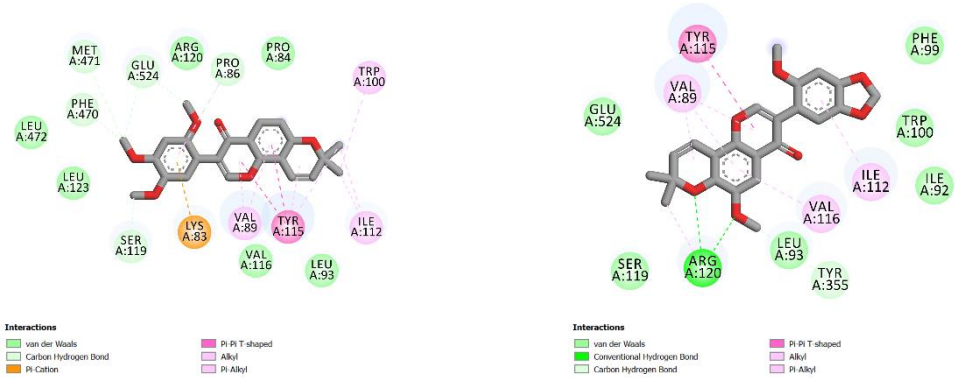
ΔG_2: Binding affinity of the investigated compound to the COX-1 enzyme (PDB ID: 6Y3C).

Considering the molecular level of potential binding of **D32**, **D19**, **D18**, **D31**, **D11**, and **D17** compounds with the COX-2 enzyme (PDB ID: 5KIR) is shown in Figure 1. The interactions between the studied compound and the enzyme mainly include van der Waals, hydrogen bonds, and non-covalent bonds such as alkyl, pi-alkyl, pi-sigma, pi-pi, and amide-pi. In particular, the **D32** compound has several hydrogen bonds with COX-2 enzyme (4 bonds) interacting with amino acids such as ASP347, PHE580, SER581, GLN192, especially amino acid residue GLN192 also interacts with Valdecoxib. Compounds **D19** and **D31** interact via hydrogen bonding with ASP120 amino acid residue in a manner similar to that with Naproxen.

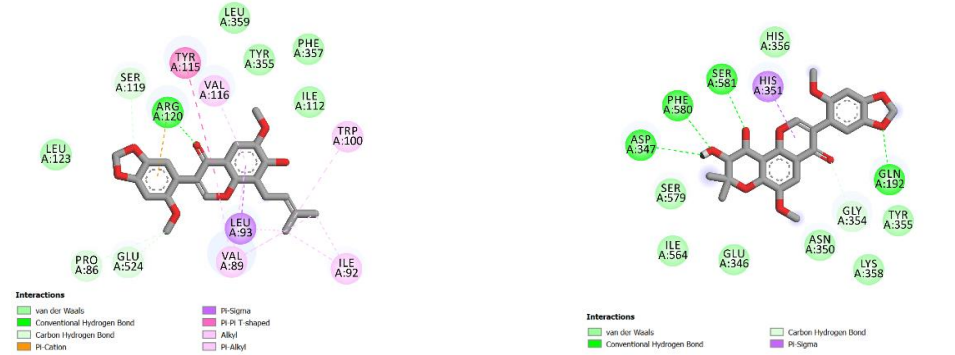


D11 *D17*

Figure 1. Potential inhibitors of the COX-2 enzyme (5KIR) identified by *in silico* screening.

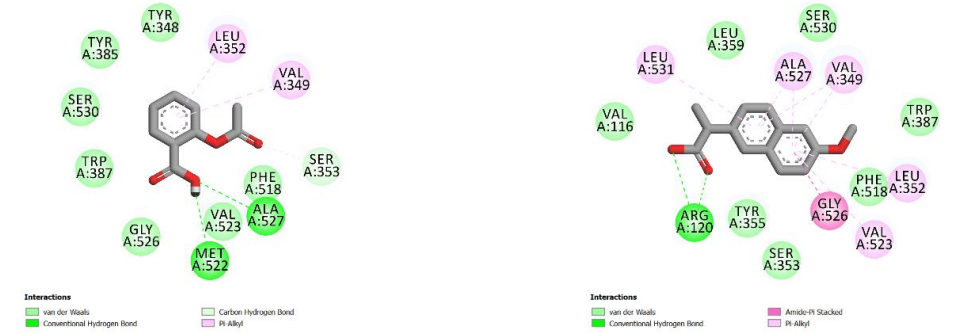


D18 *D19*



D31 *D32*

Valdecoxib *Lummiracoxib*



Aspirin *Naproxen*

Figure 1 (*continue*). Potential inhibitors of the COX-2 enzyme (5KIR) identified by *in silico* screening.

Previous studies have reported that compounds such as durallon (**D17**), barbigerone (**D18**), ichthynone (**D19**), and millesianin D (**D31**) inhibit NO production. Barbigerone (**D18)** is found to significantly inhibit the production of TNF-α [7, 28]. However, those compounds haven't been investigated for their effects on COXs. Our results suggested that the compounds mentioned above could be potential anti-inflammatory drugs by selectively inhibiting the COX-2 enzyme. *In vitro* and/or *in vivo* studies are suggested to confirm their activities.

4. CONCLUSION

By molecular docking using Autodock Vina software, millesianin E (**D32**), barbigerone (**D18**), millesianin D (**D31**), (+)-epicatechin (**D11**), durallone (**D17**), and ichthynone (**D19**) among 50 compounds isolated from *M. dielsiana* were found to exhibit potential selective binding to the COX-2 enzyme compared with COX-1. Our results may help guide *in vitro* and *in vivo* assays to find and develop anti-inflammatory drugs with fewer side effects such as gastric mucous membrane complications.

Acknowledgements. The work was completed with financial support from the Ministry of Education and Training belonging to the Ministerial Science and Technology Project, Code No. B2021-TNA-19.

CRediT authorship contribution statement. Ha NX: Formal analysis, Investigation. Le VTT: Formal analysis, Funding acquisition. Lam DT: Formal analysis. Quan PM: Methodology, Investigation. Dat VT: Formal analysis, Supervision.

Declaration of competing interest. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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