p-ISSN: 2477-3328
e-ISSN: 2615-1588
International Summit on Science Technology and Humanity (ISETH2019)
Advancing Scientific Thought for Future Sustainable Development

Anti-inflammatory Activities of Methyl *trans*-Cinnamate derivatives on Carrageenan-Induced Paw Edema in Male Sprague-Dawley Rats

Teni Ernawati[1], Tri Yuliani[1], Minarti[1], Indah Dwiatmi Dewijanti[1]

[1]Research Center for Chemistry LIPI, Kawasan PUSPIPTEK, Serpong, Tangerang Selatan, Banten- Indonesia 15314

Abstract

Purpose: The purpose of this study was to evaluate the anti-inflammatory activity of cinnamic acid and 3-phenyl propanoic acid and also virtual screening of the methyl *trans*-cinnamate derivatives were carried out on the COX-2 enzyme.

Methodology: The cinnamic acid and 3-phenylpropanoic acid were tested for anti-inflamatory activity in vivo using carageenan-induced rat paws edema method. Thirty male Sprague-Dawley rats were divided into 5 groups: control group, Na diclofenac (0.45 mg/kg body weight), ibuprofen (7.2 mg/kg body weight), cinnamic acid (14.4 mg/kg body weight) and 3-phenyl propionate acid (14.4 mg/kg body weight). Paw edema was observed using a plethysmometer for 46 hours, these were at 0, 0.5, 2, 3, 4, 5, 22, 29 and 46 hours. Each compound of methyl *trans*-cinnamate derivatives to be performed molecular docking, virtual constructed in the form of two-dimensional (2D) using BKChem application. The simulation molecular docking using PLANTS 1.1. The structure of methyl *trans*-cinnamate derivatives was characterized by IR, melting point, UV, ^{1}H NMR, ^{13}C NMR, and mass spectral analysis.

Results: The result showed that antiinflamatory activity of Na diclofenac, ibuprofen, cinnamic acid, and 3-phenil propionic acid were -20,6 %, -3,7 %, -43,2 %, 27,7 % respectively. These results showed only 3-phenil propionic acid could reduce edema in the rats' paw. The docking results show that cinnamic acid, 3-phenyl propanoic acid and 3- (4-benzoylphenyl) propanoic acid are predict to have activity as COX-2 inhibitors.

Applications/Originality/Value: methyl trans-cinnamate derivative compounds as candidates for anti-inflammatory drugs.

Keywords: *methyl trans-cinnamate derivative, antiiflammatory, COX-2, molecular docking*

INTRODUCTION SECTION

Inflammation is a manifestation of damage of cells in the tissue. The symptoms are pain, swelling, presence of inflammation, heat and redness. Patients will seek theraphy to relieve the pain, through pharmacotherapy, physiotherapy or surgery. However, simple analgesics mostly have not been able to control the pain of arthritis . (Aman, 2005).

Non Steroidal Anti-inflammatory Drugs (NSAIDs) are the most widely class of drugs used as an analgesic, anti-pyretic, and anti-inflamatory such as in arthritis. (Rosmiati et al., 1995). However, analgesic dosage often has side effects that can be fatal. Therapeutic effects and side effects of NSAIDs are associated with the mechanism of action of the drug on the enzyme cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2), interfering with the biosynthesis of prostaglandins. Prostaglandins is not only a pro-inflammatory substance, but also a gastroprotector. Therefore, selective COX-2 inhibitor is thought to be free of side effects on the gastrointestinal tract. However, selective COX-2 inhibitor is not always free of side effects, even in the gastrointestinal tract. The major concern is side efffect in the cardiovascular system. (Aman, 2005). The possible mechanism is describe below.

COX-2 activity produce prostacyclin which act as vasodilator and inhibitor of platelet aggregation. Meanwhile, COX-1 activity produce thromboxane which has opposite effect with

p-ISSN: 2477-3328
e-ISSN: 2615-1588

prostacyclin. Selective COX-2 inhibition will result in increasing blood viscosity and vasoconstriction, which lead to thrombotic events. (Adam, et al., 1999).

NSAIDs that can inhibit prostaglandins production has heterogeneous chemical structure and different pharmacodynamic. Therefore, NSAIDs can be classified based on chemical structure, acidity, pro-drug or not, and selective inhibition on COX-1 and COX-2. Efficacy of NSAIDs itself is largely determined by its ability to inhibit prostaglandin synthesis through inhibition of COX activity.

Various clinical trials in patients with osteoarthritis shown that NSAIDs, both selective and non-selective COX-2 inhibitor still have efficacy in reducing pain. (Bensen, et al. (1999) ; Ehrich, et al. (1999)). However, selective COX-1 inhibitors diminish anti-inflammatory activity. In the other hand, the more selective COX-2 inhibitor, the greater its anti-inflammatory activity. (Cannon, et al., 2000).

We tried to synthesize methyl cinnamic derivatives that have activity in inhibiting the production of prostaglandins which also selectively inhibit COX-2. There are some ways to modify the structure of methyl cinnamic in order to achieve bioactivity desired, for example by adding functional groups in the aromatic region, adding functional groups around a double bond or increase/decrease the functional groups in the ester/carboxylate. Many functional groups can be added and many processes can be done in the modification of the structure of methyl *trans*-cinnamate derivatives.

It will take a long time and require substantial funding to conduct all the experimental. Molecular docking is a technique to save time and costs so that all the experiment need not to be done. In this paper, we try to conduct molecular docking of methyl cinnamate derivative of the enzyme COX-2 to be simulated in software using a belay molecule protein-ligand systems ANT (PLANTS 1.1). The real target of methyl *trans*-cinnamate derivatives are proteins in the living body. To be able to interact with proteins, methyl *trans*-cinnamate derivatives must first enter into the active site/ mooring sites of the protein. Volume and shape of the molecule methyl *trans*-cinnamate derivatives must be in accordance with the volume and shape of the protein sites. (Motiejunas, et al.(2006); Taylor, et al.(2006)). If not, these molecules can not enter. Similarly, the group, should be in accordance with the amino acid at that site. Conformity between the protein and ligand referred to as «Lock and Key», and when this condition is met, then the compound (methyl *trans*-cinnamate derivatives) regulate the conformation of the molecule when tethered to a stable and accurate. Methyl *trans*-cinnamate derivatives predicted active in molecular docking will be tested further in vivo for antiinflammatory activity.

MATERIAL AND METHODS

Materials

The molecular docking study was carried out to explore the binding mode of methyl *trans*-cinnamate derivatives within the binding pocket of the COX-2 and to understand their structure activity relationship using PLANTS 1.1 as docking software. Compound of ZINC03814717 was used as COX-2 inhibitor reference (Figure 1) virtual targets and validated configuration file for the identification of inhibitors of COX-2 and the structure of the methyl *trans*-cinnamate derivatives compounds tested (Figure 2). (Yuniarti, et al.(2011) ; Ernawati, (2012).

3-phenyl propionic acid and cinnamic acid were obtained from the Laboratory of Synthesis Research Center for Chemistry (RCC- LIPI) Serpong. Physiological saline, Ibuprofen and

Diclofenac Na (Cataflam®) obtained from a pharmacy. Carrageenan and Tween 80 (Merck) and distilled water.

Animals Test

30 Male albino Sprague Dawley rats (*Rattus norvegicus L.*) with two months old and body weight ± 200 g were used. Rats were fed with standard pellets and distilled water ad libitum and acclimatized for 2 weeks before the test.

Apparatus

Molecular docking. Hardware: Computer with 2 GB of memory and an Intel Core i3 M380 (4 processors) @ 2.53 GHz. Software: Ubuntu Operating System 10.04, Open Babel 2.2.3, Bkchem 0.13.0, application preparation of ligands and protein for molecular docking SPORES, molecular docking was used Protein-Ligand Systems ANT (PLANTS1.1), and molecular visualization was used PyMOL 1.2r.

Anti-inflammatory activity: plestimometer, disposable syringe 1 mL and 3 mL, oral needles, tweezers, digital scales, measuring glass.

Methods

Each compound of methyl *trans*-cinnamate derivatives to be performed molecular docking, virtual constructed in the form of two-dimensional (2D) using BKChem application. Output file applications protonated at pH 7.4 BKChem and constructed the structure in three dimensions (3D) using the Open Babel application. (Yuniarti, et al., 2011). With the application of the Open Babel Similarly, the output in 3D is done 10 conformers search using Monte Carlo simulation method and teroptimal energy conformers with a maximum of 1000 steps minimized by steepest descent method (Ernawati, 2012). The structure of this optimization results were prepared using SPORES to be ready as input for simulations of molecular docking (O'Bole et al., 2011). The results of this preparation then be input to the simulation molecular docking using PLANTS 1.1. (TenBrink, et al.(2009) ; Korb, et al.(2009)). Simultaneously also performed the same procedure on ZINC03814717 as reference compounds. ChemPLP Score of the molecular docking result is compared with ChemPLP scores of the reference compound ZINC03814717. To determine whether the test compound ChemPLP scores better than reference compounds ZINC03814717 was done statistical test *1-tailed t-test* paired.

Antiinflammatory Activity

30 rats were divided into 5 groups: control group, Na diclofenac (0.45 mg/kg body weight), ibuprofen (7.2 mg/kg body weight), cinnamic acid (14.4 mg/kg body) and 3-phenyl propanoic acid (14.4 mg/kg body weight). Rats were fasted 12 hours before administering the test compounds or standard drugs. Test compounds were given orally. Thirty minutes later, the rats were challenged by a subcutaneous injection of 0.05 ml of 1% solution of carrageenan into the plantar side of the right hind paw. The paw is marked with ink at the level of the lateral malleolus and immersed in mercury up to this mark. The paw volume is measured plethysmographically immediately after injection, after 0.5, 2, 3, 4, 5, 22, 29 and 46 hours. Dose conversion from human to rats. *Natrium Diclofenac*: human dose: 25 mg, human-to-rat conversion factor: 0.018, rat dose: 25 mg x 0.018 = 0.45 mg for rat weighting 200 g. *Ibuprofen:* human dose : 400 mg, human-to-rat conversion factor: 0.018, rat dose of 400mg x0.018 = 7.2 mg for rat weighting 200 g. *Calculation of the dose of the test compound using a dose twice the dose of ibuprofen:* human dose of 400 mg, human-to-rat conversion

p-ISSN: 2477-3328
e-ISSN: 2615-1588

factor: 0.018, rat dose: 2 x 400mg x0.018 = 14.4 mg for rats weighting 200 g. (Kurumbail, et al., 1996).

RESULT AND DISCUSSION

In molecular docking of methyl *trans*-cinnamate derivatives, we use a single compound ZINC03814717 as reference compounds. Reference compounds have been able to show the best discrimination compared to other inhibitors. Also, ZINC03814717 compounds have been reported as a COX-2 inhibitor with IC_{50} value of COX-2 = 0.29 lm or pIC_{50} = 6.54, where IC_{50} COX-2 is the large concentration of COX-2 inhibitors can inhibit the activity of COX-2 by 50% while the pIC_{50} is the prediction of biological activity through docking simulations. (Kurumbail, et al. (1996); Penning, et al. (1997)).

Methyl *trans*-cinnamate derivatives are simulated on the enzyme COX-2 is among others; methyl *trans*-cinnamate, cinnamic acid, methyl 3-phenylpropanoate, 3-phenylpropanoic acid, methyl 2-bromo-3-phenylpropanoate, methyl 3-cinnamamido 4-hydroxy butanoate, *N,N*-diethylcinnamamido, 3-(4-benzoylphenyl)acrylic acid, cinnamaldehyde, 4-phenylchroman-2-one. All the above compound structure can be seen in Figure 1.

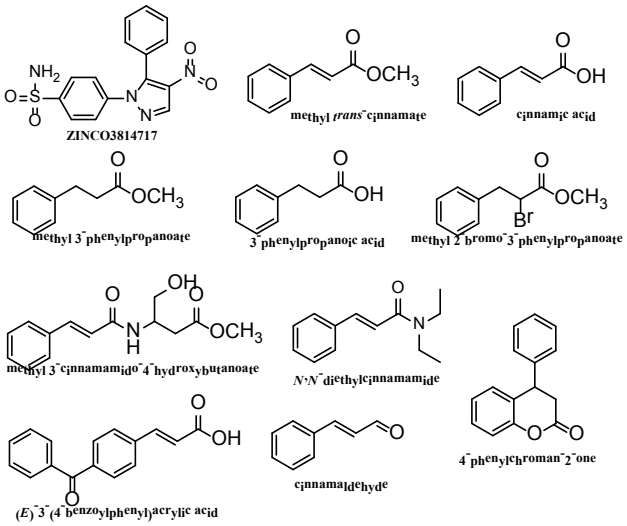

Figure 1. Methyl *trans*-cinnamate derivatives tested against the enzyme Cyclooxygenase-2 and compound ZINC03814717 as reference

Table 1. Result of molecular docking of methyl *trans*-cinnamate derivatives

Compounds	Score of ChemPLP[a]		Prediction[b]
	Sample	Reference	
Methyl *trans*-cinnamate	-79,972 ± 0,298	-89,889 ± 0,038	Not COX-2 Inhibitor
Cinnamic acid	-104,238± 0,180	-89,916 ± 0,055	Inhibitor COX-2
Methyl 3-phenylpropanoate	-80,248 ± 0,062	-89,887±0,0069	Not COX-2 Inhibitor
3-phenylpropanoic acid	-102,954± 0,145	-89,939 ± 0,047	Inhibitor COX-2
Methyl 2-bromo-3-phenylpropanoate	-76,616±1,791	-89,932 ± 0,083	Not COX-2 Inhibitor
Methyl 3-cinnamamido 4-hydroxy butanoate	-90,789 ± 1,167	-89,885 ± 0,084	Not COX-2 Inhibitor

N,N- diethylcinnamamido	-75,049 ± 1,395	-89,958 ± 0,013	Not COX-2 Inhibitor
3-(4-benzoylphenyl)acrylic acid	-107,483± 1,093	-89,984 ± 0,050	Inhibitor COX-2
Cinnamaldehyde	-73,092 ± 0,070	-89,919 ± 0,029	Not COX-2 Inhibitor
4-phenylchroman-2-one	-80,524 ± 0,631	-89,932 ± 0,079	Not COX-2 Inhibitor

[a] The value of the objective function (purata ± SD of 3 times replication) of the molecules used tethering apps.

[b] Predicted compounds active if it has a score value of the test compound ChemPLP better (smaller) than the reference compound significantly ($p < 0.05$) at 1-tailed test statistic t-tes paired with a confidence level of 95%.

The results of molecular docking methyl *trans*-cinnamate derivatives can be seen in Table 1. From the table 1, can be seen scores ChemPLP values of each compound tested was found that compounds cinnamic acid, 3-phenylpropanoic acid and 3-(4-benzoylphenyl)acrylic acid has ChemPLP score better value than the reference compounds ZINC03814717.

Molecular docking study of methyl *trans*-cinnamate derivatives result in three predicted active compound, namely cinnamic acid, 3-fenilpropionat acid and acid 3-(4-benzoylphenyl)acrylic acid. Two of them were tested for anti-inflamatory activity in vivo, using carageenan-induced rat paws edema method as described above. The volume of edema was shown in Figure 2.

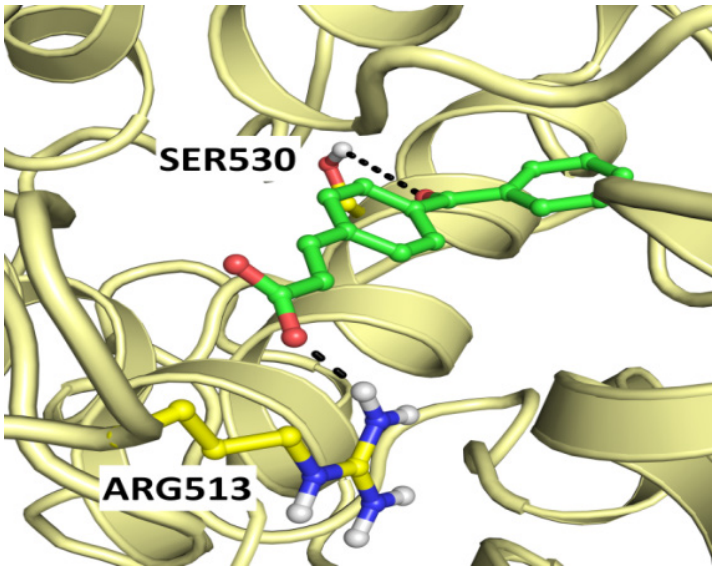

Figure 2. Molecular docking of 3-(4-benzoylphenyl)propanoate bindings pocket of COX-2. Compound of 3-(4- benzoylphenyl)propanoate was reported to hydrogen bonds form (shown as a dashed black line) with amino acid residue ARG513 and SER530.

The inflammatory activity is measured by comparing the area under the curve (AUC) of the control group vs treated groups. Anti-inflammatory activity after the administration of 3-phenylpropanoic acid, cinnamic acid and sodium diclofenac and ibuprofen groups are respectively: 27.7%, - 43.2%, - 20.6% and - 3.7. During 46 hours, only 3-phenylpropanoic acid has anti-inflammatory activity. However, during 22 hours, only cinnamic acid that does not have anti-inflammatory activity.

During 22 hours, three compounds (Na diclofenac, ibuprofen, and 3-phenylpropanoic acid) are active as an anti-inflammatory, but Na Diclofenac and Ibuprofen extend the duration of inflammation.

p-ISSN: 2477-3328
e-ISSN: 2615-1588

From the graph it can be concluded that 3-phenylpropanoic acid has the same T-max with the standard drugs but more rapid onset and longer duration of action than standard drugs (ibuprofen and Na diclofenac).

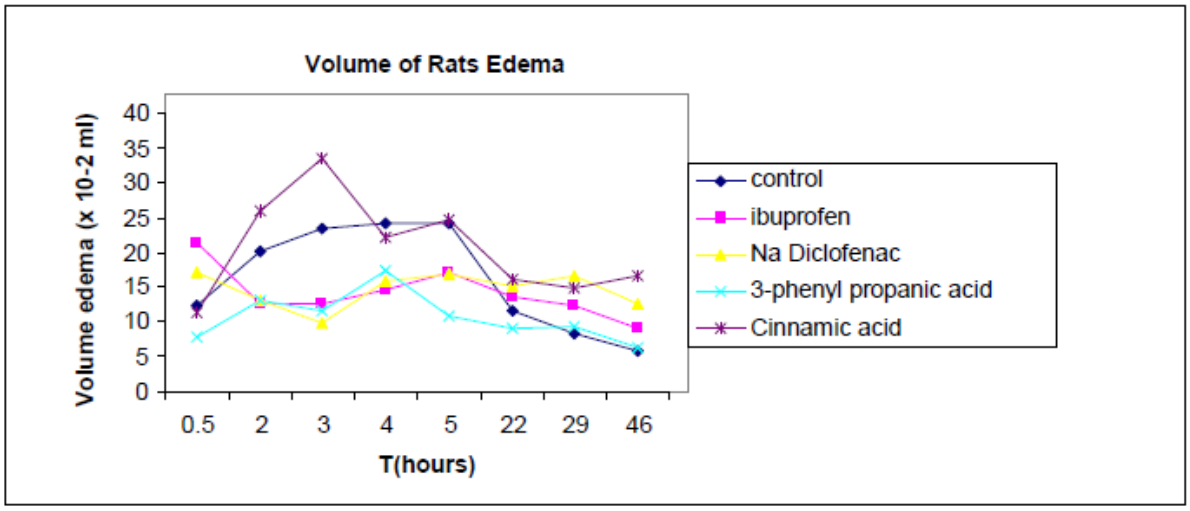

Figure 3. Relationship of mice edema volume against time

CONCLUSION

Molecular docking study result in 3 predicted active compound (of 10) because of their better ChemPLP score than reference compound (ZINC03814717). The compound cinnamic acid and 3-phenyl propanoic acid, were tested for anti-inflamatory activity in vivo. During 46 hour, only 3-phenyl propanoic acid has anti-inflammatory activity with advantages a more rapid onset and a longer duration of action than the standard drug, but equivalent activity during 3 hours.

CONFLICT OF INTEREST

We declare no conflict of interest

ACKNOWLEDGMENT

We are very grateful for the funded that were given by Competitive Program Indonesian Institute of Sciences (LIPI). In addition, also we acknowledge the support from Research Center for Chemistry – Indonesian Institute of Sciences in facilitating the research.

References:

1. Aman, K.A. (2005). Oklusi Pembuluh Darah dan Permasalahannya. Jakarta, 12-15.
2. Rosmiati, H., Gan, V.H.S. (1995). Antikoagulan, Antitrombosit, Trombolitik dan Haemostatik dalam Farmakologi dan Terapi. Edisi ke-4. Jakarta: Gaya Baru,755-762.
3. McAdam, B.F., Catella-Lawson, F., Mardini, I.A., Kapoor, S., Lawson, J.A., FitzGerald, G.A. (1999). Systemic biosynthesis of prostacyclin by cyclooxygenase (COX)-2: the human pharmacology of a selective inhibitor of COX-2. *Proc Natl Acad Sci USA*. 96, 272–277.
4. Bensen, W.G., Fiechther, J.J., McMirren, J.I., (1999). Treatment of osteoarthritis with celecoxib, a cyclooxygenase-2 inhibitor: a randomized controlled trial. *Mayo Clin Proc 74*,

1095-1105.

5. Ehrich, E.W. (1999). Effect of specific Cox-2 inhibition in osteoarthritis of the knee: a 6 week double blind placebo controlled pilot study of rofecoxib. *J Rheumatol 26,2438-2447*.
6. Cannon, G.W., Caldwell, J.R., Holt, P. (2000). Rofecoxib, a specific inhibitor of cyclooxygenase 2, with clinical efficacy comparable with that of diclofenac sodium: results of a one-year, randomized, clinical trial in patients with osteoarthritis of the knee and hip. Rofecoxib Phase III Protocol O35 Study Group. *Arthritis Rheum 43,978-987*.
7. Motiejunas, D., Wade, R.C. (2006). Computer-Assisted Drug Design, Structural, Energetics, and Dynamic Aspects of Ligand-Receptor Interactions.
8. Taylor, J.B., Triggle, D.J. (2006) *Comprehensive Medicinal Chemistry II Volume 4*, Elsevier.
9. Yuniarti, N., Ikawati, Z., Istyastono, E.P. (2011). The importance of ARG513 as a hydrogen bond anchor to discover COX-2 inhibitors in a virtual screening campaign. *Bioinformation*, *6*, *164-166*.
10. Ernawati, T. 2012. Penapisan Virtual senyawa turunan Metil sinamat pada enzim siklooksigenase-2(COX-2). Jurnal Kimia Terapan Indonesia, 14, 63-70.
11. O'Boyle, N.M., Banck, M., James, C., Morley, C., Vandermeersch, T., Hutchison, G.R. (2011). Open Babel. An open chemical toolbox. *Journal of Cheminformatics*, *3*, 33-36.
12. Ten Brink, T., Exner, T.E. (2009). Influence of protonation, tautomeric, and stereoisomeric states on protein-ligand docking results. *J. Chem. Inf. Model*, *49*, 1535-1546.
13. Korb, O., Stützle, T., Exner, T.E. (2009). Empirical scoring functions for advanced protein-ligand docking with PLANTS. *J. Chem. Inf. Model*, *49*, 84-96.
14. Penning, T.D., Talley, J.J., Bertenshaw, S.R. Carter, J.S., Collins, P.W., Docter, S., Graneto, M.J., Lee, L. F., Malecha, J.W., Miyashiro, J.M., Rogers, R.S., Rogier, D.J., Yu, S.S., Anderson, G.D, Burton, E.G., Cogburn, J.N., Gregory, S., Koboldt, C.M., Perkins, W.E., Seibert, K., Veenhuizen, W., Zhang, Y.Y., Isakson, P. C. (1997). Synthesis and biological evaluation of the 1,5-diarylpyrazole class of cyclooxygenase-2 inhibitors: identification of 4-[5-(4-methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl]benzene sulfonamide (SC-58635, celecoxib). *J. Med. Chem.*, *40*, *1347-1365*.
15. Kurumbail, R., Stevens, A., Gierse, J. (1996). Structural basis for selective inhibition of cyclooxygenase-2 by anti-inflammatory agents. *Nature*, *384*, 644-648.