

PATCHOULI ALCOHOL ISOMERS POGOSTEMON HERBA PREDICTED VIRTUALLY

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ABSTRACT

The aim of our research is predicting the alpha-patchouli alcohol isomer Pogostemon Herba as inhibitors cyclooxygenase (COX-1 and COX-2) isoenzymes. The data for the alpha-patchouli alcohol isomer (CD521903, CD442384, and/or CD6432585) Pogostemon Herba were explored from the pubchem database. Molecular interaction studies with COX-1 and COX-2 from mouse were done using the molecular docking tools Hex 6.12 and LeadIT2 Biosolve. The analysis of the alpha-patchouli alcohol compounds of patchouli oil showed that alpha-Patchouli alcohol (CD521903) binds to COX-1 at active sites including: LEU223B, ASP228B, LEU237B, ARG 332B, TRP 138A, GLU 139A, SER 142A, ASN 143A, and the interaction to COX-2 at active site including: GLN 289B, GLU 290B, ARG 222B, LYS 211B, THR 212B, HIS 214B, ASN 382B, HEM682B, GLN 454B, HIS 386B, TRP 387B, HIS 388B, VAL 274B, GLN 203B, VAL 291B, VAL 295B. The interaction hydrogen bond energy between alpha-patchouli alcohol: (CD521903-COX-1 complexes (-4 kJ/mol) and CD521903-COX-2 complexes (-8 kJ/ mol) by LeadIT2 Biosolve. This suggests alpha-patchouli alcohol CD521903 as candidate for a selective COX-2 inhibitor. These in silico data need further analyses of biological function activity.

Keywords: alpha-patchouli alcohol, COX-1/ COX-2, inhibitor selective, predicted virtually

INTRODUCTION

Secondary metabolites of patchouli plant were grouped into non-volatile compounds (Wang *et al.*, 2010) and volatile compounds (patchouli oils). alpha patchouli alcohol was the major compounds of patchouli oil (Raharjo S.J. & Retnowati R., 2012). alpha-patchouli alcohol has the effect of anti-inflammatory activity, by regulating the mRNA expression of the panel of inflammatory mediators, including TNF- α , IL-1 β , iNOS and COX-2 (Xian *et al.*, 2011). Molinspiration analysis of major compounds of patchouli oil predict activities as inhibitors for e.g, kinases, proteases and nuclear receptors (Raharjo S.J. & Fatchiyah, 2013). The data for the alpha-patchouli alcohol isomer (CD521903, CD442384, and/or CD6432585) Pogostemon Herba were explored from the pubchem database.

Cyclooxygenase enzymes exist in two isoforms, cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) (Dannhardt & Laufer, 2000). Both isoforms differ in tissue distribution and have different regulatory functions. COX-1 is a constitutive enzyme that catalyzes the regulatory prostanoid formation in various tissues, especially in mucous membranes of the gastrointestinal tract, in kidneys, platelets and vascular epithelium. In contrast, COX-2 is induced, by inflammation, mutagenesis or ontogenesis. COX-2 leads to the production of prostanoid mediators for pain and inflammation (Dannhardt & Laufer, 2000). This led to the hypothesis that COX-1 catalyzes the formation of prostaglandins for carrying out physiological regulatory functions, whereas COX-2 catalyzes the formation of "bad" prostaglandins that cause inflammation (Kartasasmita, 2002). The hypothesis explains that classic NSAID (Non Sterol Anti Inflammatory Drugs) toxicity in the gastrointestinal tract caused by drugs like acetyl salicylic acid, phenylbutazone, indomethacin, diclofenak, ibuprofen and naproxen is due to the non-selectivity for COX-1 and COX-2 (Dannhardt & Laufer, 2000).

A strategy to reduce the toxicity of classical NSAIDs could be selective inhibition of COX-2. Structurally, there are several classes of selective COX-2 inhibitors, namely: (1) derivatives carboxylic and heterocyclic to bound vicinal with moieties aril,

(2) derived diaryl- or aryl /heteroaryl- ethers and thioethers, (3) cis-stilbene derivative, and (4) diaryl ketones and aryl/ heteroaryl. Until the year 2000 about 500 compounds have been synthesized that are selective COX-2 inhibitors (Dannhardt & Laufer, 2000). Celecoxib[®] and Rofecoxib[®] are compounds, which have passed clinical trials and are on the market. In patients with osteo- and rheumatoid arthritis, selective COX-2 inhibitors exhibit anti-inflammatory function which is equivalent to the classical NSAIDs, but with milder toxicity in the gastrointestinal tract. However, an increase in blood pressure was also reported as a side effect of selective COX-2 inhibitors (Kartasasmita, 2002). Even though selective COX-2 inhibitors are milder drugs, care has to be taken to monitor and avoid potential side effects.

Virtual modeling can be used as a preliminary analysis prior to functional tests enzyme inhibitors. Therefore, we screened the alpha-patchouli alcohol isomer compounds from patchouli oil using their structures from the pubchem database docking tools with COX-1 and COX-2 to visualize molecular interactions. Virtual modeling can be used as a preliminary analysis prior to in vitro analysis of enzyme inhibitor COX conformation.

MATERIAL AND METHOD

Patchouli Oil

Alpha-patchouli alcohol isolated from patchouli oil. Patchouli oil obtained from the steam distillation of Pogostemon Herba (Pogostemon cablin Benth) for 8 hours (Raharjo S.J. & Yusuf AY, 2012) and isolation of alpha-patchouli alcohol compounds of patchouli oil by using column chromatography (Tsai *et al.*, 2007). The identification of major compounds was accomplished by using the Gas Chromatography-Mass Spectrometric (GC-MS) to major compounds analysis with GC-MS Shimadzu QP-2010S, column Rtx-5MS, length 30 meters, and Wiley8 library (Raharjo S.J., Retnowati R., & Soebiantoro, 2012).

Retrieval of Target Sequence

3D model was obtained from SWISS-MODEL reposi-

tory for cyclooxygenase-1 (COX-1) ID: EDL_08676.1 (<http://swissmodel.expasy.org/repository>) and 3D model from PDB ID : 6COX for cyclooxygenase-2 (COX-2) ID: EDL_39487 (Zukhrullah & Aswad, 2012).

Ligand Preparation

Alpha-patchouli alcohol compounds was downloaded from pubchem.ncbi.nlm.nih.gov (<http://pubchem.ncbi.nlm.nih.gov>) then its energy form were minimized and converted to PDB-format by Open Babel 2.3.1 (<http://openbabel.org/>) as ligand for virtual screening.

Docking Ligand – Protein

We used the rigid docking by Hex 6.12 software (<http://hex.loria.fr>) to compute possible interaction COX-1 and COX-2 with alpha-Patchouli alcohol isomer (CD521903, CD442384 and CD6432585) on its interaction site. Output of rigid docking were refined using IntelLigand-LigandScout 2.02 software (<http://ligandscout.software.informer.com>) to perform Identification of Van Der Walls Interaction and LeadIT2 software. And also, we used LeadIT2 software to simulate most possible native complex structure of alpha-Patchouli alcohol isomer

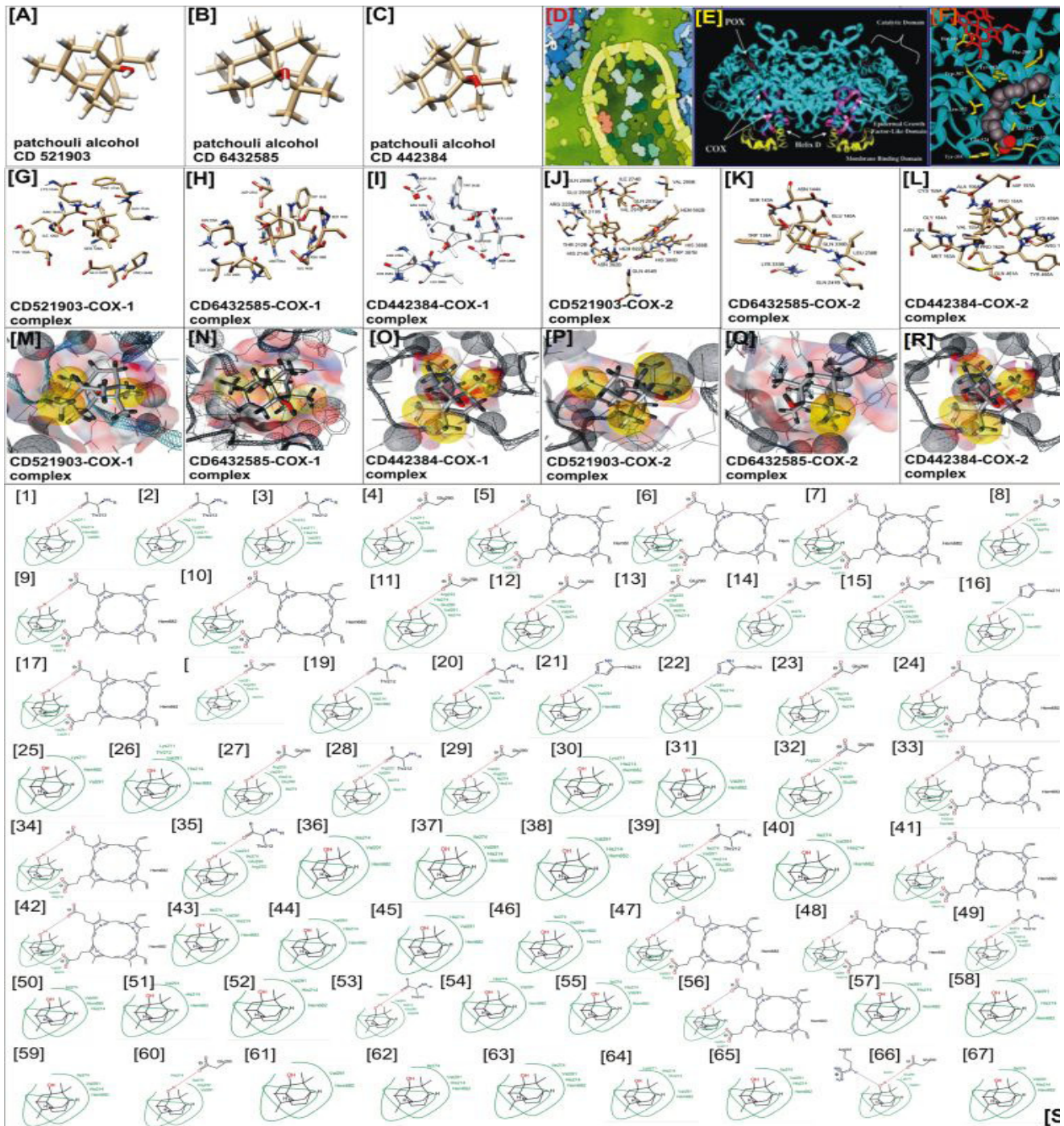


Figure 1. Modeling analyses suggest that alpha-Patchouli alcohol isomers binds to COX-1 and COX-2. [A-C] 3D Structure alpha-patchouli alcohol isomers (CD 521903, CD6432585, and CD442384); [D] COX enzymes in action (Goodsell D.S, 2000) ; [E] Structural representation of COX-1 dimer [10]; [F] Arachidonic acid bound in the active site of oCOX-1 (Goodsell D.S., 2000) ; [G-I], [J-L] Modeling analysis suggest that alpha-Patchouli alcohol (CD521903/ CD6432585 / CD442384) binds to COX-1 and COX-2, we used by Hex 6.12 Docking Software and Chemira 1.7 Viewer Software. [M-O] [P-R] Modeling analysis suggest that alpha-Patchouli alcohol (CD521903/ CD6432585 / CD442384) binds to COX-1 and COX-2, we used by LigandScout Software. [S] Modelling 2D Hydrogen Bond and energy analyses of alpha-Patchouli alcohol (CD 521903) binding to cyclooxygenase-2, used by LeadIT 2.1.3 Docking and Viewer Software.

(CD521903, CD442384 and CD6432585) respectively binds with COX-1 and COX-2 in flexible mode, which were include both backbone and side-chains movements. Thereafter, we used LeadIT2 refines the candidate models according to an energy function and hydrogen bond analysis.

Visualization

All the visualization of the structure files were done using Chemira 1.7s molecular graphics system.

RESULT

The Patchouli plant (*Pogostemon cablin* Benth) was obtained from the Pujiharjo-Tirtoyudo, Malang and the plants was carried out determination in Plant Anatomy Laboratory, Biology Department, Brawijaya University. Results of determination patchouli plants was *Pogostemon cablin* Benth, Variety of Sidikalang (Raharjo S.J. & Retnowati R., 2012). We produced the patchouli oil distillation on P_{inlet} 1,0 - 1,1 atm and P_{outlet} 0,9 - 1,0 atm. We identified that alpha-patchouli alcohol using Gas Chromatography- Mass Spectrometric (GC-MS) on patchouli oil on 6th fraction as shown Table 1.

We used the Hex 6.12 (rigid docking) to compute possible interaction COX-1 and COX-2 with alpha-Patchouli alcohol isomers (CD521903, CD643285 and CD442384) on its interaction site. The data are represented by chemira 1.7s software in Figure 1 (G-I and J-L). Next, van der Waals interactions were calculated by Portable InteLigand-LigandScout 2.02 Software (Figure 1 (M-O and P-R), Furthermore, hydrogen bonding energy was analysed by LeadIT2 software and is shown in Figure 1 (S). The results for all complexes with COX-1 and COX-2 are summarized in Table 2.

DISCUSSION

The major compounds of patchouli oil were alpha-patchouli alcohol (MW = 222 g/mol) and alpha-bulnusenene, seychellene, alpha-guaiene with MW = 204 g/ mol were respectively (Raharjo S.J. & Retnowati R., 2012). The result GC-MS analysis of alpha-patchouli alcohol on patchouli oil on 6th fraction unidentified as single compounds, but these compounds have several isomers. Some of these isomers (CD521903, CD442384 and CD6432585) were using as ligand and these ligands were obtained from pubchem database (<http://pubchem.ncbi.nlm.nih.gov/summary>) as shown Figure 1 (A-C).

Table 1. Characteristic of Patchouli Oil Component and Fraction of Column Chromatography of Patchouli Oil using analysis of GC-MS Shimadzu QP 2010

No.	Retention Time	Patchouli Oil (% relative)					Molecular Weight (g/mol)	Group
		Patch Oil	4 th fraction	6 th fraction	7 th fraction	Compounds		
1.	12,828	5,05	6,63	-	-	alfa-gurjunene	204	Sesquiterpenoid
2.	13,360	3,49	5,27	-	-	beta-caryophyllene	204	Sesquiterpenoid
3.	13,573	19,42	25,12	-	-	alfa-guaiene	204	Sesquiterpenoid
4.	13,798	9,25	13,20	-	-	seychellene	204	Sesquiterpenoid
5.	13,977	9,15	12,68	-	-	alpha-patchoulene	204	Sesquiterpenoid
6.	14,075	1,68	1,88	-	-	gemacrene-D	204	Sesquiterpenoid
7.	14,411	3,71	5,17	-	-	gemacrene-A	204	Sesquiterpenoid
8.	14,537	21,17	28,22	-	-	alfa-bulnusenene	204	Sesquiterpenoid
9.	16,501	-	-	-	25,84	viridiflorol	222	Sesquiterpenoid alcohol
10.	16,755	1,17	33,60	100,0	74,16	patchouli alcohol	222	Sesquiterpenoid alcohol

Table 2 Analysis of Virtual Modelling of COX-1/ COX-2-sesquiterpenoid/ sesquiterpenoid alcohol complexes.

No.	Virtual Modelling	Active Site (by Hex 6.12 and Chemira 1.7s)		Van Der Waals (by Ligand Scout) Σ Globe		Hydrogen bond energy (by LeadIt2) Σ interaction		Energy of Hydrogen Bond kJ/ mol (by LeadIt2)	
		COX-1	COX-2	COX-1	COX-2	COX-1	COX-2	COX-1	COX-2
A	Sesquiterpenoid alcohol								
1.	Patchouli Alcohol CD521903	TRP 138A, GLU 139A, SER 142A, ASN 143A LEU223B, ASP228B, LEU237B, ARG 332B	GLN 203B, LYS 211B, THR 212B, HIS 214B, ARG 222B, VAL 274B, GLN 289B, GLU 290B, ASN 382B, HIS 386B, TRP 387B, HIS 388B, VAL 291B, VAL 295B, GLN 454B, HEM 682B	3	4	113	67	-4	-8
2.	Patchouli Alcohol CD643285	TRP 141B, GLU 142B, SER 145B, ASN 146B, ASP 231A, ASN 239A, LEU 240A, GLN 243A, ARG 335A	TRP 139A, GLU 140A, SER 143A, ASN 144A, LEU 238B, GLN 241B, GLN 330B, LYS 333B,	4	2	nd	147	nd	-8
3.	Patchouli Alcohol CD442384	TRP 141B, GLU 142B, SER 145B, ASN 146B, ASP 231A, ASN 235A, LEU 240A, GLN 243A, ARG 335 ^a	ASN 39A, ARG 150A, PRO 154A, VAL 155A, ALA 156A, CYS 159A, ASP 157A, MET 163A, PRO 162A, GLY 164A, LYS 459A, TYR 460A, GLN 461 ^a	4	2	nd	nd	nd	nd

In Figure 1 (D), cyclooxygenase enzyme as showed red color was found in the smooth endoplasmic reticulum and in the nuclear membrane. The enzyme extracts arachidonic acid as showed orange color directly from the membrane and folds it in half, forming a five member ring at the bend and decorating it with peroxides. The resultant molecule was released, and then is modified by the peroxidase active site, which faces into the endoplasmic reticulum. Several additional enzymes then add finishing touches, and the mature prostaglandins (yellow V-shaped molecules) diffuse out of the cell and on to their target (Goodsell, 2000). Structural representation of COX dimer, as showed in Figure 1 [E]. The N-terminal epidermal growth factor domain is designated in pink and leads into the four R-helices of the membrane binding domain (yellow). Helix D projects up into the COX active site, which is located at the base of the large, globular catalytic domain (cyan). The heme prosthetic group (red) lies in the POX active site (Blobaum & Marnett, 2007). In Figure 1[F] as shown arachidonic acid bound in the active site of COX-1. The carboxylate of the substrate ion-pairs with Arg-120 and hydrogen-bonds with Tyr-355 at the crowding site, projects up the hydrophobic channel, and makes an L-shaped bend around Tyr-385. The heme prosthetic group is designated in red. Residues that are in contact with arachidonic acid in the active site channel are shown in yellow (Blobaum *et al.*, 2007). COX-1 and COX-2 are two distinct isoforms of cyclooxygenase, and plays a vital role in conversion of arachidonic acid to prostaglandin. Prostaglandins are involved in various pathological processes like inflammatory responses, carcinogenesis and cardiovascular events (Amaravani, Prasad, & Ramakrishna, 2012). These theoretical data suggests alpha-patchouli alcohol (CD521903, CD442384, CD6432585) as potential inhibitor of COX-1 (Raharjo S.J. & Fatchiyah, 2013). These virtual predictive can be used preliminary selective or non selective COX-1 and COX-2.

Rigid docking to compute possible interaction COX-1 and COX-2 with alpha-Patchouli alcohol isomers (CD521903, CD643285 and CD442384) on its interaction site and the interaction result, as shown on Figure 1 (G-I and J-L), van der Waals interaction, as shown on Figure 1 (M-O and P-R) and hydrogen bonding energy, as shown in Figure 1 (S). The results for all complexes with COX-1 and COX-2 are summarized in Table 2.

In silico analysis was predicting patchouli oil compounds as candidate ligand receptor to using cyclooxygenase-1 (COX-1 or PGH1 mouse) and cyclooxygenase-2 (COX-2 or PGH2 mouse). All isomers have ability as inhibitor COX-1 and COX-2, but the best modeling analyses suggest that alpha-patchouli alcohol isomer compounds of patchouli oil was alpha-patchouli alcohol (CD521903-COX-1 complexes and CD521903 COX-2 complexes). Modeling analysis suggest that alpha-Patchouli alcohol (CD521903) binds to COX-1 at active sites including: LEU223B, ASP228B, LEU237B, ARG 332B, TRP 138A, GLU 139A, SER 142A, ASN 143A, and the interaction to COX-2 at active site including: GLN 289B, GLU 290B, ARG 222B, LYS 211B, THR 212B, HIS 214B, ASN 382B, HEM682B, GLN 454B, HIS 386B, TRP 387B, HIS 388B, VAL 274B, GLN 203B, VAL 291B, VAL 295B, as shown Figure 1 [S]. We conducted interaction hydrogen bond energy (with none model solvent) between alpha-patchouli alcohol: CD521903-COX-1 complexes (-4 kJ/mol) and CD521903-COX-2 complexes (-8 kJ/mol). In silico analysis will suggest the ability patchouli alcohol CD521903 as inhibitor cyclooxygenase-2 selective novelty.

Modeling analysis suggest that alpha-Patchouli alcohol (CD521903) binds to COX-2 at active site including: GLN 289B, GLU 290B, ARG 222B, LYS 211B, THR 212B, HIS 214B, ASN 382B, HEM682B, GLN 454B, HIS 386B, TRP 387B, HIS 388B, VAL 274B, GLN 203B, VAL 291B, VAL 295B, so that complex formation CD521903-COX-2 would blocking oxygenated reaction and peroxides reaction currently arachidonic acid substrate into the enzyme COX-2. When compared of establishment with the COX-1-CD521903 complexes, CD521903-COX-2 complexes over selective as COX-2 inhibitors. Also supported, hydrogen bond energy (None Model Solvent) CD-521903-COX-1 complexes (-4 kJ/mol) was smaller than the CD-521903-COX-2 complexes (-8 kJ/mol) by LeadIt2 software. Collectively, our results suggest that alpha-Patchouli alcohol (CD-521903) is suggesting an inhibitor of COX-2 selective by LeadIt2 software. Further in silico analysis using molecular dynamic virtual by Amber12 software. And also in silico data await conformation by biological activity analysis.

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