Asian Journal of Chemistry; Vol. 23, No. 7 (2011), 3093-3096

ASIAN JOURNAL OF CHEMISTRY

www.asianjournalofchemistry.co.in

Analyzing the Interaction of Shellegueain A: A Bioactive Compound of Pakis Tangkur (*Selliguea feei* or *Polypodium feei*) to Cyclooxygenase Enzyme by Molecular Docking

ALIYA NUR HASANAH*, JUTTI LEVITA, ELFANDARI DEWI NATAPOERA and ANAS SUBARNAS

Faculty of Pharmacy, Universitas Padjadjaran, Jl Raya Bandung Sumedang Km 21,5 Jatinangor, West Java, Indonesia

*Corresponding author: Fax: +62 227796200; Tel: +62 8122346382; E-mail: aliya_nh@yahoo.com

(*Received*: 21 September 2010; *Accepted*: 12 March 2011) AJC-9735

Shellegueain A is an active compound contained in pakis tangkur (*Selliguea feei* or *Polypodium feei*). This compound has been proven to show analgesic activity by decreasing writhing response in acetic acid-induced rats. It also showed antiinflammatory activity by significantly reducing oedema in carrageenan-induced rat's paw. The purpose of this study is to examine the binding modes of shellegueain A against COX-1 and COX-2 in terms of hydrogen bonds and docking energy, to understand its analgesic and antiinflammatory properties. The simulation indicated that shellegueain A did not interact with either COX-1 or COX-2 enzymes, while afzelechin (a monomeric metabolite of shellegueain A) did by making hydrogen bonds with Met522.

Key Words: Cyclooxygenase, Shellegueain A, Afzelechin, Selliguea feei, Molecular docking.

INTRODUCTION

Pakis tangkur, (*Selliguea feei*), which can be found wildly grown at Tangkuban Perahu Mountain in West Java, Indonesia, has been empirically used as a pain reducer. Shellegueain A, a novel sweet trimeric proanthocyanidin with a double-linked A units, is a bioactive compound of this plant. The structure of this substance was established as epiafzelechin-(4β → 8, 2β → O → 7)-epiafzelechin-(4β → 8)-afzelechin[1]. Afzelechin is the monomer subunit of shellegueain A (Fig. 1).

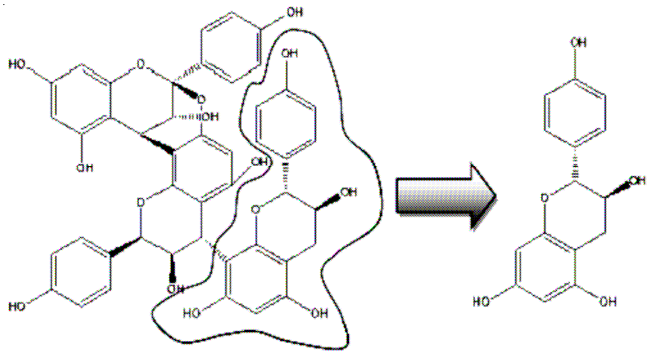


Fig. 1. 2D structures of shellegueain A (left) and afzelechin (right)

Cyclooxygenase (COX) plays an important role in inflammatory response. This enzyme has been analyzed by X-ray crystallography at a resolution of 3.0 Å and visualized as a homodimer with 587 amino acid residues per chain thus yielding a molecular weight of 67230 daltons[2]. Two isoforms of the cyclooxygenase enzyme, which are COX-1 and COX-2, exist. These two isoforms share a sequence identity of 60 % denoting that the overall structures of the enzyme isoforms are highly conserved. The overall structures of COX-1 and COX-2 are highly conserved although COX-2 was shown to have a much larger non-steroidal antiinflammatory drug binding site due to the substitution of a valine for isoleucine (Fig. 2) at position 523 in the active site[3]. The cyclooxygenase

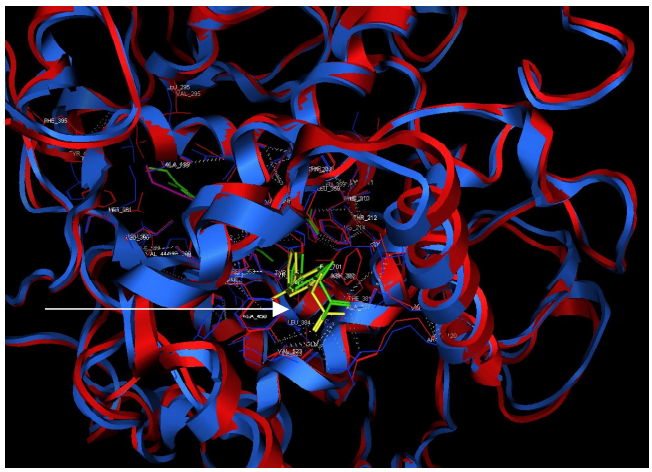


Fig. 2. Alignment of COX-1 (red) and COX-2 (blue) with flurbiprofen co-crystalized in both enzymes. White arrow shows two molecules of flurbiprofen (coloured in green and yellow) which are located at the same site in the binding pocket of both enzymes

active site contains Tyr355, Tyr385, Ser530, Arg120 and Val349. The most important amino acid is Tyr385 which catalyze the transformation of arachidonic acid to PGG2[4].

The interaction of shellegueain A and its monomer metabolite, afzelechin, with cyclooxygenase enzyme was studied using molecular modeling technique, *e.g.*, molecular docking. The docking result was compared with flurbiprofen, a nonselective inhibitor of COX enzyme.

EXPERIMENTAL

A Windows VistaTM Home Basic (2007) computer with Genuine Intel Core Duo T2060 1,60 GHz, 80 GB, ATI Radeon Xpress 200M Series and RAM 1.5 GB capacity of memory, was prepared for computational study in this work.

The X-ray crystallographic 3D structures of COX-1 (PDB code: 1EQH) and COX-2 (PDB codes: 3PGH) were downloaded from online Protein Data Bank (http://www.rcsb.org/pdb).

General procedure

Molecular modeling: 2D and 3D structures of shellegueain A, afzelechin and flurbiprofen were built using ChemOffice 2004 programme (downloaded from www.cambridgesoft.com). Energy minimization of each molecule was carried out by using AM1 method with Polak-Ribiere algorithm from Portable HyperChem Release 8.0.7 programme (downloaded from http://www.hyper.com). The programme was also applied to calculate the ligands' QSAR properties.

Macromolecule preparation: The X-ray crystallographic 3D structures of COX-1 (PDB code: 1EQH) and COX-2 (PDB codes: 1CX2 and 3PGH) were downloaded from online Protein Data Bank (http://www.rcsb.org/pdb/). Hydrogens were added to all COX enzymes PDB crystal structures followed by calculating their partial charges. SwissPDBViewer v.4.01 (GlaxoSmithKline R&D, downloaded from http://www.expasy.org was used to separate the monomer of the macromolecules.

Ligand-protein docking: Ligand-protein docking was applied to understand the molecular interaction of shellegueain A with COX-2 and COX-1 enzyme. Docking was simulated with AutoDockTools v3.05 in MGLTools v1.5.2 (Molecular Graphics Laboratory, The Scripps Research Institute) downloaded from http://mgltools.scripps.edu).

The interaction between shellegueain A and afzelechin with both of the COX enzymes was analyzed and compared with flurbiprofen and acetosal, nonselective inhibitors of COX enzyme.

RESULTS AND DISCUSSION

2D structures of shellegueain A and afzelechin which were built by using ChemOffice 2004 and calculated as shown in Fig. 1 and Table-1 informed that shellegueain A was very hydrophilic (c log P value = -7.06) due to its many hydroxyl moieties hence made this compound difficult to be absorbed. On the contrary, shellegueain A's monomer, afzelechin, shows different features. The latter two compounds have sufficient hydrophobicity which makes them absorbable.

TABLE-1
ANALYSIS OF LIGANDS

Compound	Shellegueain A	Afzelechin	Flurbiprofen
Energy (kcal mol^{-1})	-10,919.89	-3,756.42	-3,474.79
c log P	-7.06	-2.09	3.39
Volume ($Å^3$)	1,864.01	759.02	726.25
Mass (amu)	816.77	274.27	244.27

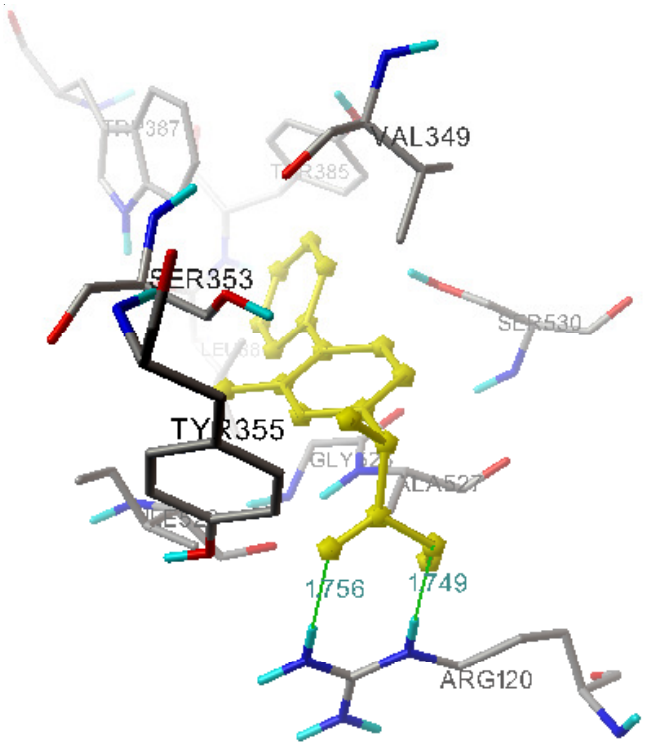
Flurbiprofen binds to Arg120 and Tyr355 in both COX-1 and COX-2 binding pockets (Table-2). RMSD values are 0.53 Å for COX-1 and 0.98 Å for COX-2, which means that the docking method is valid[5,6]. In COX-1 binding pocket, this compound forms two hydrogen bonds with Arg120 at 1.756 and 1.749 Å, respectively, while in COX-2 it interacts with Arg120 at 1.747 Å and with Tyr355 at 1.832 Å (Fig. 3).

Docking of shellegueain A into COX-1 and COX-2 binding pocket showed that this compound could not interact spontaneously (docking energy has positive values). These results are due to the volume of shellegueain A. The volume of cyclooxygenase enzyme binding site is 8 Å × 25 Å[7], while the volume of shellegueain A is larger, 14.298 Å × 12.559 Å.

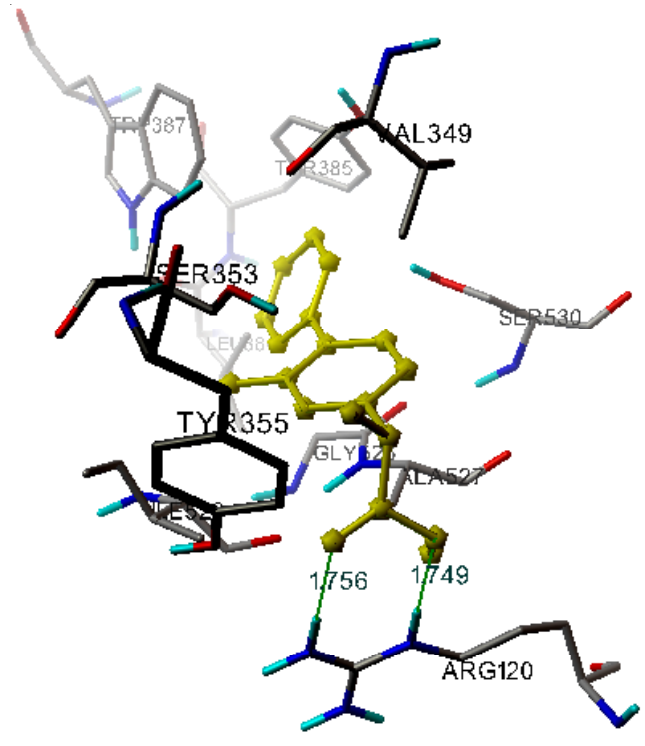
Afzelechin, which size is 6.705 Å × 11.919 Å, was docked into the binding pocket of COX-1 and COX-2 enzymes as shown in Fig. 4.

TABLE-2
TOP SCORE DOCKING OF FLURBIPROFEN AND SHELLEGUEAIN A INTO COX-1 AND COX-2

Compound	Flurbiprofen		Shellegueain A	
	COX-1	COX-2	COX-1	COX-2
Docking energy (kcal/mol)	-9.95	-9.90	433.76	375.7
Gibbs energy (kcal/mol)	-9.86	-9.81	433.53	375.56
Inhibition constant (nM)	59.9	64.7	–	–
Hydrogen bond	O-FLP → NH2-Arg120 O-FLP → HE-Arg120	O-FLP → H-Tyr355 O-FLP → NH_2-Arg120	–	–
VDW[d]	Arg120, Val349, Tyr355, Ile523, Gly526, Ala527, Ser530, Leu384, Tyr385, Trp387	Val349, Tyr355, Met522, Gly526, Ala527, Tyr385, Trp387, Ser535	Phe198, Phe205, Val344, Tyr348, Val349, Leu352, Tyr355, Tyr385, Trp387, Phe518, Met522, Ile523, Gly526, Ala527, Ser530, Leu531, Leu534, Ser535	Val89, His90, Leu93, Val116, Arg120, Val349, Leu352, Tyr355, Arg513, Val523, Glu524, Gly526, Ala527, Leu531, Ser535



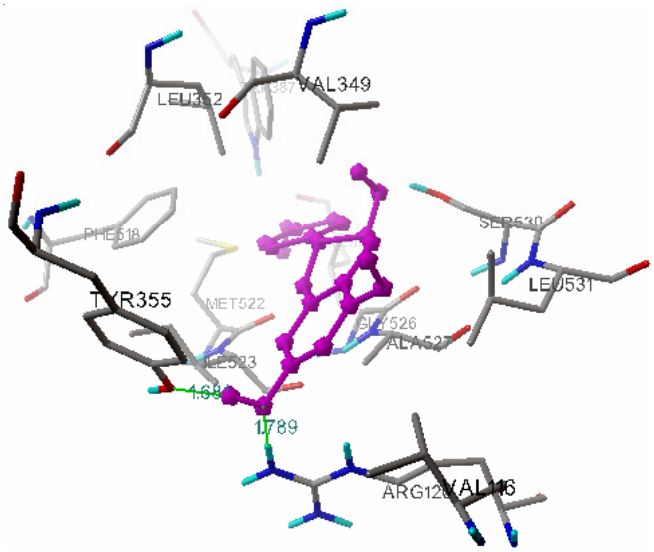
(a)



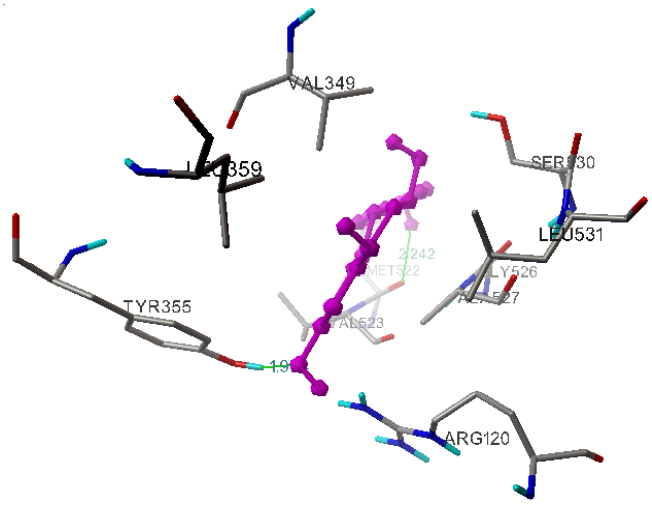
(b)

Fig. 3. Docking of flurbiprofen into the binding site of (a) COX-1 (b) COX 2. Flurbiprofen is visualized by yellow ball and stick model. Green lines indicates hydrogen bonds which are formed between flurbiprofen and the amino acids in the binding pocket of COX-1 and COX-2

Docking energy afzelechin into COX-1 and COX-2 resulted negative values, which meant that afzelechin interacted spontaneously with cyclooxygenase enzymes (Table-3). In COX-1 binding pocket, this compound forms one hydrogen bond with Arg120 at 1.682 Å and with Tyr355 at 1.789 Å, respectively, while in COX-2 it only interacts with Tyr355 at 1.92 Å (Fig. 4).



(a)



(b)

Fig. 4. Docking of afzelechin into the binding site of (a) COX-1 and (b) COX-2. Afzelechin is visualized by magenta ball and stick model. Green lines indicates hydrogen bonds which are formed between afzelechin and the amino acids in the binding pocket of COX-1 and COX-2

Afzelechin formed hydrogen bond with Met522. This type of interaction can inhibit the binding of arachidonic acid to Tyr385, which explains and confirms the analgesic and anti-inflammatory activity of shellegueain A at *in vivo* assay as previously studied and published[8].

Conclusion

Shellegueain A did not interact either with COX-1 or COX-2 binding sites. Afzelechin that was assumed as a metabolite and monomer of shellegueain A interacts with COX-1 and COX-2 enzyme *via* hydrogen bond formation with Met522.

TABLE-3
TOP SCORE DOCKING OF FLURBIPROFEN AND AFZELECHIN INTO COX-1 AND COX-2

Compound	Flurbiprofen		Afzelechin	
	COX-1	COX-2	COX-1	COX-2
Docking energy (kcal/mol)	-9,95	-9,90	-9,06	-8,69
Gibbs energy (kcal/mol)	-9,86	-9,81	-9,02	-8,64
Inhibition constant (nM)	59,9	64,7	244	462
Hydrogen bond	O-FLP → NH_2-Arg120 O-FLP → HE-Arg120	O-FLP → H-Tyr355 O-FLP → NH_2-Arg120	H-AFZ → O-Tyr355 O-AFZ → H-Arg120	Val116, Val349, Leu352, Tyr355, Leu359, Met522, Ile523, Gly526, Ala527, Ser530, Tyr385, Trp387, Ser535
VDW[d]	Arg120, Val349, Tyr355, Ile523, Gly526, Ala527, Ser530, Leu384, Tyr385, Trp387	Val349, Tyr355 Met522, Gly526, Ala527, Tyr385, Trp387, Ser535	O-AFZ → H-Tyr355 H-AFZ → O-Met522	Val116, Val349, Tyr355, Leu359, Met522, Val523, Gly526, Ala527, Ser530, Leu531

REFERENCES

1. N.I. Baek, M.S. Chung, L. Shamon, L.B. Kardono, S. Tsauri, K. Padmawinata, J.M. Pezzuto, D.D. Soejarto and A.D. Kinghorn, *J. Nat. Prod.*, **56**, 1532 (1993).
2. G.F. Fabiola, L. Damodharan, V. Pattabhi and K. Nagarajan, *Curr. Sci.*, **80**, 26 (2001).
3. R.G. Kurumbail, A.M. Stevens, J.K. Gierse, J.J. McDonald, R.A. Stegeman, J.Y. Pak, D. Gildehaus, J.M. Miyashiro, T.D. Penning, K. Seibert, P.C. Isakson and W.C. Stallings, *Nature*, **384**, 644 (1996).
4. http://www.chem.umass.edu/~cmartin/Courses/BioStruct/2005/StudentPresentations/KristenHuber/W_KristenHuber.pdf
5. G. Jones, P. Willett, R.C. Glen, A.R. Leach and R. Taylor, *J. Mol. Biol.*, **267**, 727 (1997).
6. I. Halperin, B. Ma, H. Wolfson and R. Nussinov, *Protiens*, **47**, 409 (2002).
7. N.V. Chandrasekharan, H. Dai, K.L. Roos, N.K. Evanson, J. Tomsik, T.S. Elton and D.L. Simmons, *Proc. Natl. Acad. Sci. (USA).* **99**, 13926 (2002).
8. A. Subarnas and H. Wagner, *Phytomedicine*, **7**, 401 (2000).