

## Docking Simulation of Fevicordin into Human Receptor Estrogen $\alpha$ \*

**<sup>1</sup>Muchtaridi**, **<sup>1</sup>Jerry Voldo F.M.**, **<sup>2</sup>Abdul Mutalib**, **<sup>1</sup>Jutti Levita**

<sup>1</sup>Faculty of Pharmacy, Universitas Padjadjaran, Bandung

<sup>2</sup>Radioisotope and Radiopharmaca Centre of BATAN, Serpong

### ABSTRACT

*Fevicordin have similar structure to estradiol and cytotoxic in nature, thus can be developed as cancer drug. Docking method can be applied to simulate it interactions with human estrogen receptor  $\alpha$  which is known involve in growth of breast cancer. Information from simulation can be used to optimize its activity on target. Docking method was validated with redocking of x-ray estradiol structure and modelled estradiol into x-ray estradiol cognate binding pocket. Fevicordin 3D structure modelled with molecular mechanic in MM+ force field, then its unique conformers modelled with molecular dynamic simulation in same force field. Each conformers docked into x-ray estradiol binding pocket (SP). Alternative pocket (AP) was searched using Q-SiteFinder software and each of fevicordin's conformers was docked into that pocket. Validation of docking method showed that AutoDock was valid to predict binding mode of x-ray estradiol and modelled estradiol and value of both predicted  $K_i$  was in prediction range. Modelled fevicordin was a planar conformation relative to estradiol and it has three conformers which were K1, a planar form, K2 and K3, both were bend form. Conformer K2 bind well into SP relative to K1 and K3 with predicted binding free energy -9,80 kcal/mol and  $K_i$  65,8 nM. Alternative pocket (AP) volume was 460 Å<sup>3</sup> and located in groove formed by helix 3, helix 5 and loop 1 of hER $\alpha$  LBD. Conformer K3 bind well into AP relative to K1 and K2 with predicted binding free energy -8,48 kcal/mol and  $K_i$  612 nM. Binding affinities comparison of fevicordin at SP and AP showed that fevicordin's binding tendency into AP, thus fevicordin was predicted unable to compete with estradiol.*

*Key word : Fevicordin, estrogen receptor, docking, estradiol*

\* Presented at International Symposium of Molecular Target, UGM, Yogyakarta, March 26, 2008

## Introduction

Fevicordin is steroidal compound isolated from mahkota dewa seed (*Phaleria marcocarpa* (Scheff) Boerl.) and had anticancer activity. The previous research, fevicordin compound was isolated from mahkota dewa (*Phaleria macrocarpa* Scheeff Boerl) seeds. The compound have cytotoxicity activity. IC<sub>50</sub> value of fevicordin on Hela cell, CASKI, and TE-2 cell were 1,25 µg/mL, 12 µg/mL, and 40 µg/mL, respectively (Diantini, *et al.*, 2007). On the other hand, fevicordin had cytotoxicity on T47D (IC<sub>50</sub> < 0.1 µg/ml) and on MCF7 (38.81 µg/ml) cell lines.

However, besides that compound is very toxic on normal cell of fibroblast (Diantini *et al.*, 2007). Looking at the structure, this compound has –OH groups in C-2 position and what make it different is other estradiol derivative is the core structure of estradiol bounded in 3-heptenone (Figure 1.). estrogen receptor (ER) ligand pharmacophore which were hydroxy group substituted aromatic ring (Duax *et al.*, 1985) was present in this compound indicated ER binding ability. Its potentiality to cure breast cancer. ER $\alpha$  was known to involve in breast cancer growth (Fujita *et al.*, 2003).

One of the problem in this research is fevicordin has specificity and selectivity to estrogen receptor which is same or stronger if it is compared to estradiol, therefore further toxicity of this compound can be used as estradiol agonist to kill cancer cell in mammary tissue.



Figure 1. Structure of estradiol (a) and fevicordin from mahkota dewa seeds (b)

## Materials and Methods

### Materials

Ligand binding domain 3D structure, 2,9 angstrom of wildtype ER $\alpha$ , cocrystallized with estradiol by Eiler et al (2001), obtained from Protein Data Bank online database ([www.rcsb.org](http://www.rcsb.org)) with access code 1G50. Fevicordin structure obtained from Diantini (Diantini *et al.*, 2007).

### Tools

We used hardware to calculate in molecular modeling and molecular docking method was using Intel® Pentium™ Dual Core Duo ([www.intel.com](http://www.intel.com)) personal computer, with Seagate 40 GB harddisk ([www.seagate.com](http://www.seagate.com)) and 2 pieces of Kingston RAM each 512 MB memory ([www.kingston.com](http://www.kingston.com)), Hyperchem™ v7.0 for Windows (Hyperchem, Inc) was used in estradiol and fevicordin modeling and its conformers, Swiss-PdbViewer v3.7 (GlaxoSmithKline R&D, [www.expasy.org](http://www.expasy.org)) was used to visualize and repairing damage structure of macromolecule and separate ligand structure from macromolecule structure. Software packet of AutoDock Tools v1.4.4 (Molecular Graphics Laboratory, The Scripps Research Institute) was used to prepare protein structure, ligand structure, grid parameter file and docking parameter file. Autogrid v3.0.5 was used to prepare grid, AutoDock v3.0.5 was used in docking simulation in cygwin environment. Q-Site Finder

was used to predict location and volume of binding pocket. OpenBabel v2.1.0 was used to convert HIN file format into PDB file format.

## **Methods**

### **Docking Method Validation**

Cocrystallized estradiol was separated from chain A of LBD ER $\alpha$ , polar hydrogens, partial charge and solvation parameter was assigned to chain A LBD ER $\alpha$  while hydrogen, partial charge was assigned to ligand; non polar hydrogens was merged. X-ray estradiol was docked into LBD ER $\alpha$  with run variation: 10, 30, 50, 70 run in  $(60)^3$  Å each grid dimension, and  $(40)^3$  Å,  $(60)^3$  Å,  $(80)^3$  Å,  $(100)^3$  Å grid dimension variation in 50 run each. Docking method valid if RMSD equal or less than 2 Å (Ali *et al.*, 2007).

### **Fevicordin modeling**

Fevicordin's 3D structure built with Hyperchem v7.0 then minimized in MM+ force field to 0,1 kcal/mol convergence through Polak-Ribiere algorithm. Hyperchem format file of this model was converted into PDB file format with OpenBabel.

### **Fevicordin's conformers modeling**

Fevicordin's model heated in 100 fs from 0 K to 300 K then assigned to 300 K's dynamic molecular simulation for 4000 fs. Potential energy and total energy of system saved in 1 fs range. Conformers was sampled in each 100 fs then saved in separated file without energy minimization. Conformers was grouped based on torsional angle C10-C9-C17 and uniqueness ring B and C. Lowest energy conformers from each group was saved in separated file. Those conformers then called fevicordin's unique conformers. Conformers file format then converted into PDB file format with OpenBabel.

### **Docking simulation of unique conformer into x-ray estradiol binding pocket**

Unique conformers and LBD ER $\alpha$  prepared using same procedure in validation procedure. Grid was built in former binding pocket with dimension  $(70)^3$  Å to covered all important residues involved in binding process. AutoGrid 3.0.5 was used to generate grid maps of oxygen, polar hydrogen, aromatic and alifatic carbon. One hundred run of Lamarckian Genetic Algorithm was used to dock each of fevicordin unique conformers into LBD ER $\alpha$  thus most of ligand conformation in binding pocket could observed. Docking result saved in dlg file then analyzed to get informations about ligand orientation, binding energy value and Ki value.

### **Alternative binding pocket searching**

Alternative pocket searching was done with QSite Finder online ([www.bioinformatics.leeds.ac.uk/qsitefinder/](http://www.bioinformatics.leeds.ac.uk/qsitefinder/)). ER $\alpha$  PDB file 1G50 chain A without estradiol was used as searching input. Search result are biggest predicted pockets and volume after steroid pocket which would used in docking simulation.

### **Docking simulation of fevicordin conformers to alternative pocket (AP)**

Conformers of fevicordin and LBD ER $\alpha$  chain A without estradiol was prepared with same procedure of logand and macromolecule preparing in method validation.. Grid dimension was 92 Å x 50 Å x 52 Å in coordinate 92,647, 24,014, 24,852 thus could cover all residues of alternative pocket. Grid maps of aromatic and alifatic carbon, osygen and polar hydrogen generated by AutoGrid 3.0.5. Docking was done using Lamarckian Genetic Algorithm in 100 runs. Docking result was saved in dg file then ligand orientation, ligand-macromolecule interactions, Ki value of top ranking conformers was analyzed and compared to experimental data of LBD ER $\alpha$ -estradiol complex.

## Results and discussion

### Docking method validation

Estradiol binded to LBD hER $\alpha$  through van der waals interactions with Leu387, Phe404, Met388, Leu428, Met421, Ile424, Leu525 on ring A, B and D, hydrogen bond formed between oxygen OH3 and hydrogen NH Glu353 ((2,87 Å), oxygen OH3 with hydrogen NH Arg394 (2,72 Å) and nitrogen NH His524 (2,71 Å). All residues mentioned above form a pocket with volume 459 Å<sup>3</sup>.

Estradiol x-ray docking into its original binding pocket with run and grid dimension variations showed that AutoDock could predict estradiol x-ray original binding mode accurately with RMSD in all variation equal or less than 2 Å. Residue Phe404 and estradiol interaction can not identified in all run variation because their closest carbon distance is larger than 3,9 Å, even though, there are additional interaction between estradiol and Leu478 in 10 runs and 70 runs. Figure 1 showed example of x-ray estradiol docking with run variation.

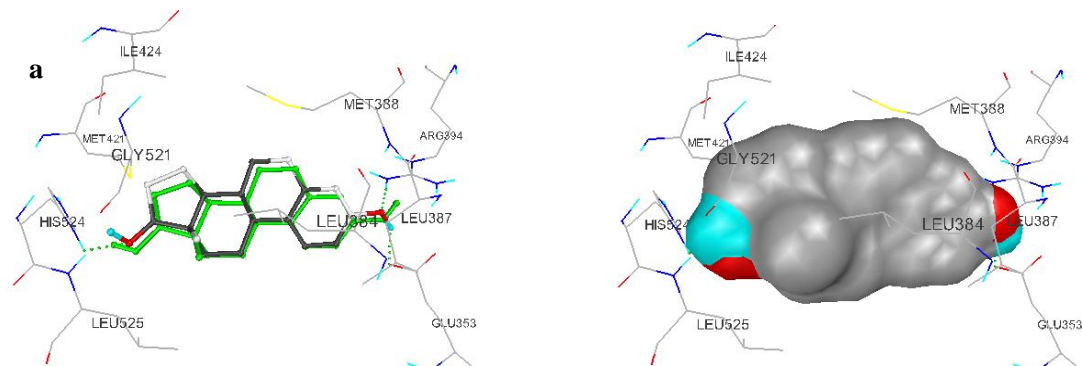


Figure 1 (a) (right) Binding mode of estradiol at 30 runs. Estradiol *x-ray* colored green, (left) visualization of molecular surface with AutoDock Tools.

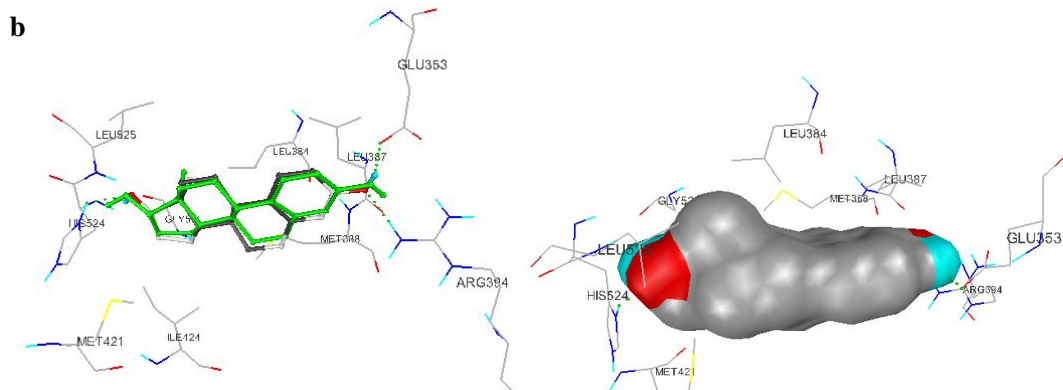


Figure 1 (b) (right) Binding mode of estradiol at grid dimension (80)<sup>3</sup> Å, *x-ray* estradiol colored green, (left) visualization of molecular surface with AutoDock Tools.

Table 1. Docking summarize x-ray estradiol at 50 runs and grid dimension (80)<sup>3</sup> Å

	FE <sup>a</sup>	Ki <sup>b</sup> (nM)	Hydrogen bond			RMSD <sup>c</sup>
			Atom EST	Residu aa <sup>d</sup>	Distance	
Run 50	-11,32	5,04	H3-EST	O Glu353	2,15	0,73
			O3-EST	HN Arg394	1,81	
			O17-EST	HN His524	2,01	
Grid Dimension (80x80x80) Å	-11,32	5,02	H3-EST	O Glu353	2,13	0,77
			O3-EST	HN Arg394	1,82	
			O17-EST	HN His524	2,07	
EST <sup>e</sup>	-10,48 <sup>f</sup>	20,7 <sup>f</sup>	H3-EST	O Glu353	2,87	
			O3-EST	HN Arg394	2,72	
			H17-EST	NH His524	2,71	

<sup>a</sup> Free energy (kcal/mol)

<sup>b</sup> Inhibition constant

<sup>c</sup> Root mean square deviation

<sup>d</sup> Amino acid residues

<sup>e</sup> x-ray estradiol bind to hER $\alpha$  LBD (PDB code:1G50)

<sup>f</sup> Docking  $\Delta$ G value calculated with AutoDock without docking simulation

All additional interaction seems to decrease free binding energy compared to free binding energy calculated with AutoDock without docking process. All hydrogen bond distance less than 3 Å thus agree with good hydrogen bond proposed by Halperin (Halperin *et al.*, 2002). This result showed that Lamarckian Genetic Algorithm using in AutoDock 3.0.5 are efficient and effective to predict true binding mode of estradiol in grid dimension that cover all important residues with proper algorithm run amount, besides that in all variation, RMSD value are equal or less than 2 Å, then AutoDock are valid in docking simulation (Ali *et al.*, 2007).

In grid dimension variation, most of true estradiol interactions can be observed with RMSD equal or less than 2 Å and increase of grid dimension equal to increase of binding free energy but doesn't significant because average of increase is 0.02 kcal/mole compare to binding affinity. In grid dimension (80)<sup>3</sup> interactions with Met421 doesn't observed. Those result showed that docking simulation with AutoDock was valid (Ali *et al.*, 2007). Example of docking result at 50 runs and grid dimension (80)<sup>3</sup> Å listed in Table 2.

Table 2. Docking summarize modeled estradiol at 50 runs and grid dimension  $(80)^3 \text{ \AA}$

	EB <sup>a</sup>	Ki <sup>b</sup> (nM)	Ikatan hidrogen			RMSD <sup>c</sup>
			Atom EST	Residu aa <sup>d</sup>	Jarak	
Jumlah Run 50	-11,02	8,37	H3-EST	O Glu353	2,17	0,65
			O3-EST	HN Arg394	1,85	
			O17-EST	HN His524	2,12	
Dimensi Grid (80x80x80) $\text{\AA}$	-11,08	7,52	H3-EST	O Glu353	1,87	0,54
			O3-EST	HN Arg394	1,83	
			O17-EST	HN His524	1,88	
EST <sup>e</sup>	-10,48 <sup>f</sup>	20,7 <sup>f</sup>	H3-EST	O Glu353	2,87	
			O3-EST	HN Arg394	2,72	
			H17-EST	NH His524	2,71	

<sup>a</sup> Free energy (kcal/mol)

<sup>b</sup> Inhibition constant

<sup>c</sup> Root mean square deviation

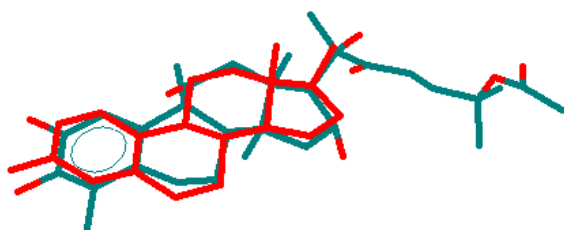
<sup>d</sup> Amino acid residues

<sup>e</sup> *x-ray* estradiol bind to hER $\alpha$  LBD (PDB code:1G50)

<sup>f</sup> Docking  $\Delta G$  value calculated with AutoDock without docking simulation

### Fevicordin and its conformers modeling

Fevicordin model was planar relative to estradiol shown in Figure 3, but there are bending in ring B and ring C junction because of substituent at C11. The angle of C10-C9-C17 is  $-32,135^\circ$  while estradiol have angle  $13,503^\circ$ . Total energy of fevicordin model is 82,854 kcal/mole.



Dynamic molecular simulation is used to modeled fevicordin conformers because similar structure, which is estradiol 11K $\beta$ , have four conformers using same method (Wiese dan Brooks, 1994). One of the predicted conformers of this estradiol analog is crystallography conformers. It is known that ER ligand structure affects its binding affinity and estrogenic activity (Palomino *et al.*, 1994, Wiese dan Brooks, 1994). Fevicordin have three unique conformers, coded K1, K2 and K3. K1 is relative planar (Figure 4 (a)) to K2 and K3 which are bended form with "L" like structure (Figure 4 (b) and Figure 4 (c)).

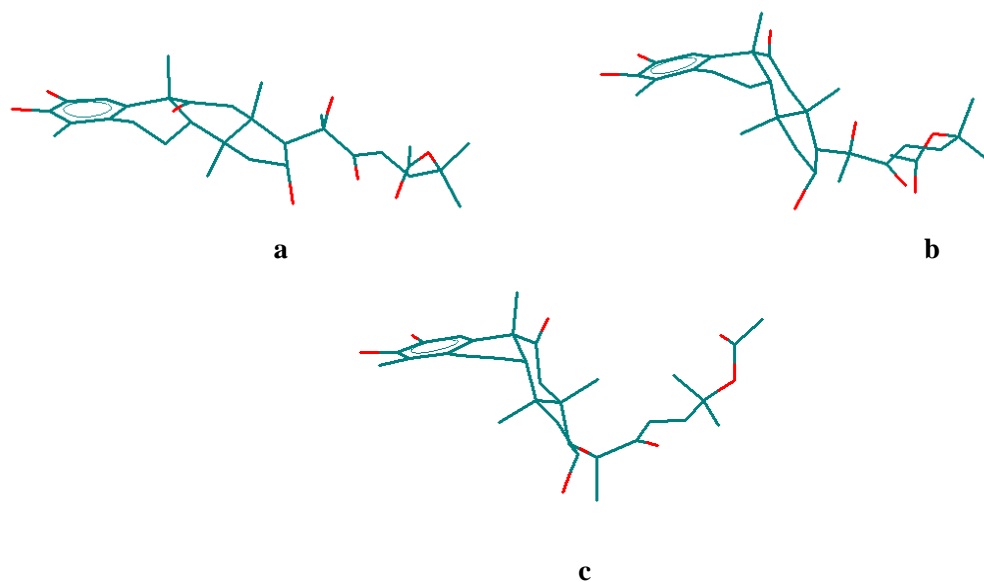


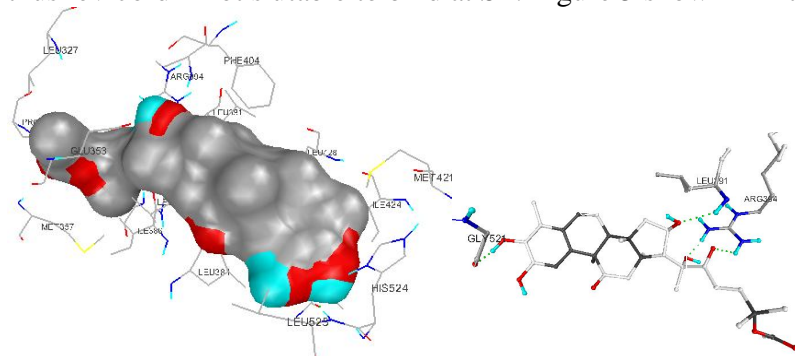
Figure 4 (a) Conformation of K1, (b) Conformation of K2, (c) Conformation of K3.

Steroid bending on K2 and K3 caused by electrostatic interactions between C11 carbonyl group which have negative charge and C1 hydrogen atom which have positive charge.

#### Docking simulation of fevicordin's unique conformation in steroid pocket (SP)

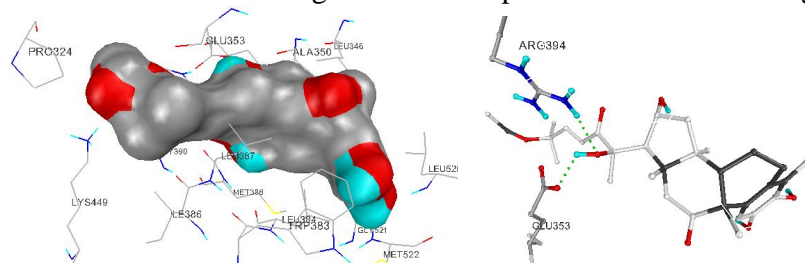
Docking simulation of K1 on SP showed inverse orientation relative to estradiol (Figure 5). This orientation caused by inability of SP to accommodate ring D's side chain of conformers, there are many steric clash in this region, such as O28 with Pro324 (2,75 Å) and OH20 (1,79 Å), C22 (2,37 Å) with Glu353, OH2 with Leu525 (2,55 Å), C28 with Ile424 (2,88 Å), OH16 with Leu387 (2,03 Å), because its shape match with "benzene" like ring (Brzozowski *et al.*, 1997). Conformers K1 formed four hydrogen bond with SP residues, listed in Table 3.

Binding free energy of K1 was predicted -2,85 kcal/mole with  $K_i$  value 0.01 M thus fevicordin not suitable to bind at SP. Figure 5 show K1 interaction at SP.

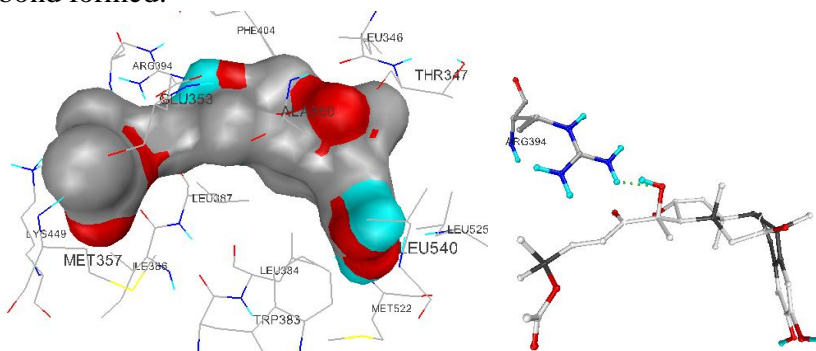


Conformers K2 bind with same orientation to K1. Steric clashes occur between K2 and residues of SP, such as Leu525 with oksigen OH2 (2,55Å), Ala350 with karbon

C11 (2,39 Å), Arg 394 with carbonyl oxygen 22 (2,62 Å), side chain of ring D suffer same steric clash like K1. This steric clash show that complementary of SP with K2 is not good, even though, affinity of K2 is better than K1. Conformers K2 formed two hydrogen bond listed in Table 3. Figure 6 showed position of K2 in binding pocket.



Conformer K3 have same orientation and steric clashes relative to K2 but only one hydrogen bond formed because of slight shift of conformer relative to K2, thus decrease its stability in SP. Figure 6 showed K3 position in SP, Table 3 listed hydrogen bond formed.



K <sup>a</sup>	K <sub>i</sub> (nM)	EB <sup>b</sup>	Residu-residu yang berkontak dekat	Ikatan hidrogen (donor→akseptor)
K1	10 <sup>7</sup>	-2,85	Gly521, Pro324, Ile386, Ile424, Leu387, Lys449, Met357, Leu384, Leu525, Met421, Glu353, His524, Phe404, Leu391, Arg394, Met388, Leu428	OH3→Gly521 (1,646 Å) Leu391→OH16 (1,646 Å) Arg394→O20(1,711 Å) Arg394→O22(1,754 Å)
K2	65,8	-9,80	Leu384, Leu387, Leu391, Phe402, Leu525, Gly521, Ile386, Arg394, Glu353, Trp383, Met388, Leu346, Met522, Lys449, Gly390, Pro324, Ala350	O23→Glu353 (1,94 Å) Arg394→H23 (2,22 Å)
K3	812	-6,94	Arg394, Leu387, Leu391, Met357, Thr347, Gly521, Leu346, Trp383, Ile386, Leu384, Phe404, Gly390, Met522, Glu353, Leu540, Leu525, Lys449, Ala350.	Arg394→OH16 (2,15 Å)

<sup>a</sup> Fevicordin conformer

<sup>b</sup> Binding free energy(kcal/mol)



## Conclusion

AutoDock was valid in this docking simulation with RMSD 2 Å. Fevicordin was planar relative to estradiol and predicted to bind at alternative pocket in binded conformation (K3).

## Reference

- Diantini, A.; D. Kurnia; A. Faried; L.S. Faried; A. Subarnas; T.H. Achmad; H. Hayashi; Supriyatna. 2007. Antiproliferative activity on hela and CASKI cells of fevicordin A isolated from the seeds of *Phaleria macrocarpa*. Proceeding International Conference On Traditional Medicine And Medicinal Plant. Surabaya 8-9 September 2007.
- Ali, H. I. *et al.*, 2007. Antitumor studies. Part 3: Design, synthesis, antitumor activity, and molecular docking study of novel 2-methylthio-, 2-amino and 2-(N-substituted amino)-10-alkyl-2-deoxo-5-deazaflavins. *Bioorganic & Medicinal Chemistry*.
- Brzozowski *et al.* 1997. Molecular basis of agonism and antagonism in the oestrogen receptor. *Letters to Nature* 389:753-757.
- Anstead, Gregory M., Kathryn E. Carlson, John A. Katzenellenbogen. 1997. The Estradiol Pharmacophore: Ligand structure-estrogen receptor binding affinity relationships and a model for the receptor binding site, *Steroids* 62:268-303.
- Duax W.L., J.F. Griffin, C.M Weeks, K.S. Korach. 1985. Molecular Conformation, Receptor Binding, and Hormone Action of Natural and Synthetic Estrogens and Antiestrogens. *Environmental Health Perspectives* 61:111-121.
- D'Ursi, Pasqualina, Erika Salvi, Paola Fossa, Luciano Milanese, Ermanna Rovida. 2005. Modelling The Interaction Of Steroid Receptors With Endocrine Disrupting Chemicals, *BMC Bioinformatics* 6(Suppl 4):S10.
- Fujita *et al.*, 2003. Full Activation of Estrogen Receptor  $\alpha$  Activation Function-1 Induces Proliferation of Breast Cancer Cells. *The Journal of Biological Chemistry* Vol 278. 29:26704-26714
- Good Sell, David S., Garrett M. Morris and Arthur J. Olson. 1996. Automated *Docking* of Flexible Ligands: Application of AutoDock, *Journal of Molecular Recognition* 9:1-5.
- Hsieh, Robert W. *et al.*, 2006. Identification of Ligands with Bicyclic Scaffolds Provides Insights into Mechanisms of Estrogen Receptor Subtype Selectivity. *The Journal of Biological Chemistry* 281(26):17909-17919.
- Ikeda, Kazuhiro and Satoshi Inoue, 2004. Estrogen receptor and their downstream targets in cancer *Arc Histol Cytol* 67(5):435-442.
- Jeyakumar *et al.*, 2007. Raloxifene And ICI 182,780 Increase Estrogen Receptor Alpha Association with a Nuclear Compartment via Overlapping Sets Of Hydrophobic Amino Acids in AF2 Helix 12. *Molecular Endocrinology*
- Katzenellenbogen, J.A., and B. Katzenellenbogen. 2000. Estrogen Receptor Transcription and Transactivation Estrogen Receptor Alpha and Estrogen Receptor Beta: Regulation by Selective Estrogen Receptor Modulators and Importance in Breast Cancer. *Breast Cancer Res* 2:335-334.
- Katzenellenbogen, John A., ‡, Rajeev Muthyala, and Benita S. Katzenellenbogen, 2003. Workshop 1.4. Nature of the ligand-binding pocket of estrogen receptor  $\alpha$  and  $\beta$ : The search for subtype-selective ligands and implications for the prediction of estrogenic activity. *Pure Appl. Chem.* 75:2397-2403.
- Kroemer, R.T. 2003. Molecular modelling probes: *docking* and scoring, *Biochemical Transactions* 31:980-984

- Lambrinidis, George *et al.*, 2006. The Estrogen receptor and polyphenols:molecular simulation studies of their interaction, a review. *Environ Chem Lett* 4:159-174.
- Morris *et al.* 1998. Automated *Docking* Using a Lamarckian Genetic Algorithm and an Empirical Binding Free Energy Function. *Journal of Computational Chemistry* 19:1639-1662.
- Nilsson, Stefan, *et al.*. 2001. Mechanism of Estrogen Action. *Physiological Reviews*. Vol 81 No.4.