Docking study of Cyclomulberrin, Cyclomorusin and Engeletin into p50 *NF-kβ* Transcription Factor with AutoDock Vina

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Abstract

Molecular docking is a comprehensive method to predict noncovalent binding of small molecules (ligand) of elucidated active compounds to final receptor. The binding show possibility of metabolism of ligand related to physiology and action of isolated chemical to the receptor. Docking of three ligand, i.e. cyclomulberrin, cyclomorusin, and engeletin with *NF-kβ* (which act as receptor/protein target), has been done to study its binding ability for description of biochemical activity interactions. The active compounds were isolated from the leave of *Artocarpus altilis* (known as breadfruit), and the structures are readily download from National Center for Biotechnology Information (NCBI) Pubchem. *NF-kβ* is the receptor responsible for the regulation and immune response. The research is using *PyRx* and AutoDock Vina method control software. The result showed that each ligand has binding affinity to *NF-kβ* receptor, with the highest score for Engeletin (-8,96 kcal/mol), followed by followed by cyclomorusin (7,59 kcal/mol) and cyclomulberrin (-7,00 kcal/mol). We conclude, that these three ligand could be proposed as candidate for further research in the inflammation field.

Keywords : *Molecular Docking, Cyclomulberrin, Cyclomorusin, Engeletin, NFkβ, PyRx, AutoDock Vina.*

Background

Artocarpus altilis (known as breadfruit) is used traditionally as phytoherbal of some diseases. It contains several active flavonoid chemical, which is three of it are cyclomulberrin, cyclomorusin and engeletin where its structure already elucidated [2,3]. Studies conducted on breadfruit extracts have revealed many potentioal properties of the plant. The leaves are used to treat liver disease and fevers in Taiwan, an extract from the flowers was effective in treating ear edema, bark extracts exhibited strong cytotoxic activities against leukemia cells in tissue culture, extracts from roots and stem barks showed some antimicrobial activity against Gram-positive bacteria and may have potential use in treating tumors [9].

NF-kβ is nuclear multifactoral found in many types of animal cells, that has many different transcription, for example as immune respone/inflamation (immunoregulatory proteins and cytokines), *NF-kβ* regulation (rel/Ikβ proteins), proliferation (cyclins, growth factors) and apoptosis/cell survival (regulators of apoptosis) [7]. The specific structure to form *NF-kβ* p50 have been identified as homodimer [4] as indexed on Research Collaboratory for Structural Bioinformatics (RSCB) protein database [5].

Molecular docking is a computational procedure that predict noncovalent binding obtained from simulations or homology modeling of macromolecules as receptor and a small molecule (ligand) efficiently, starting with their unbound structures. Ligand is examined, according to its isolated chemical structure and characterization, which show its ability to affect and give influence to a receptor. Bound conformation and binding affinity were obtained as result of simulation [1]. This research is aimed to investigate the ability of cyclomulberrin, cyclomorusin and engeletin as ligand to bind into target macromolecule i.e. *NF-kβ* receptor, by using PyRx docking software with pre-installed AutoDock Vina [1]. It will show a brief description of metabolizing ability of ligand into target molecule/receptor.

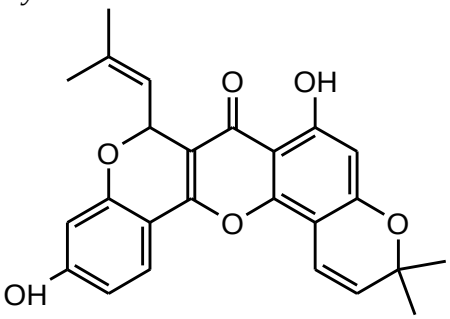
Methods

Ligands data and preparation

The database of three flavonoids structure used in molecular docking studies, were derived as isolated from breadfruit

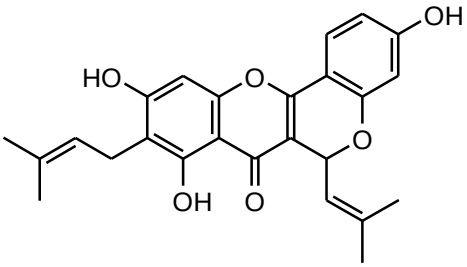
(*Artocarpus altilis*) [3] and downloaded from National Center for Biotechnology Information (NCBI) Pubchem [6]. The chemical and physical important data of the three flavonoids and its geometric drawing are

- *Cyclomorusin* (NCBI, 2005) [11] o Molecular Weight : 418,43858 g/mol o Molecular Formula : $C_{25}H_{22}O_6$
  - ○ CID : 5481969
  - ○ Synonyms : *Cyclomorusin A, Cyclomulberrochromene*



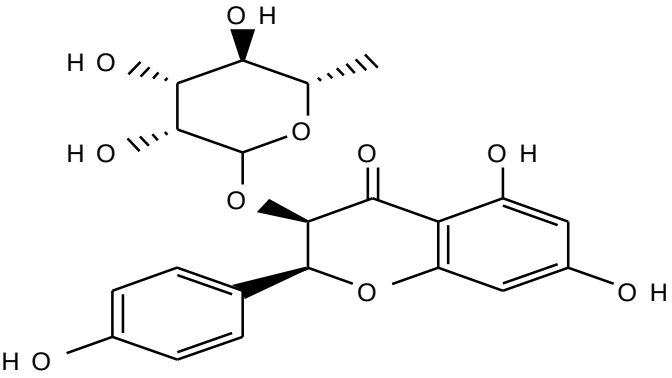
Picture 1. Two dimensional structure of *cyclomorusin*.

- *Cyclomulberrin* (NCBI, 2006) [12] o Molecular Weight : 420,45446 g/mol o Molecular Formula : $C_{25}H_{24}O_6$ o CID : 11742872
  - ○ Synonyms : -



Picture 2. Two dimensional structure of *cyclomulberrin.*

- *Engeletin* (NCBI, 2006)[13] ○ Molecular Weight : 434,39338 g/mol ○ Molecular Formula : $C_{21}H_{22}O_{10}$
  - ○ CID : 6453452 ○ Synonyms : *Engelitin*

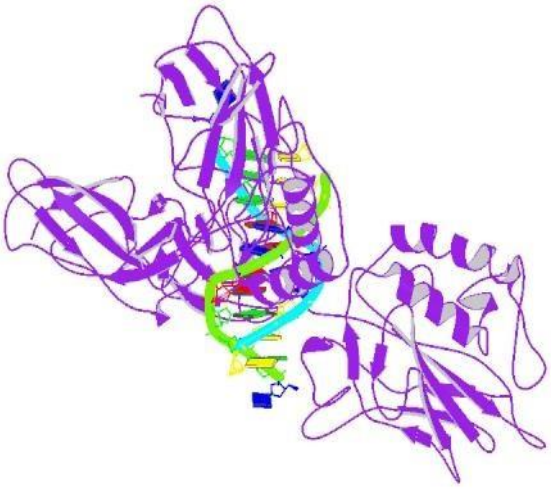


Picture 3. Two dimensional structure of *engeletin*.

Structure Data File (SDF) format file of ligand structures were retrieved from NCBI PubMed [14], and by using Open Babel tool, .sdf file is converted to .pdbqt file [15] and then to .pdb file for subsequent *PyRx* docking [16].

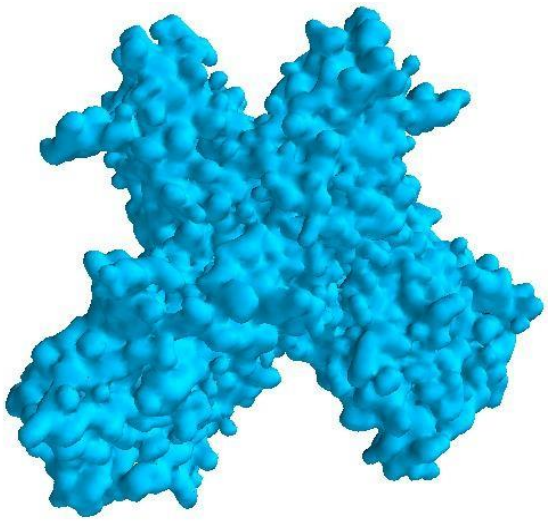
The database of *NF-kβ* is three dimensional (3D) structure. The structure that used for this experiment was retrieved from protein data bank, with PDB code was 1NFK. Depend of the download site, the structure consist complex homodimer p50 *NF-kβ*-DNA[4]. *NF-kβ* will act as protein target or receptor.

Properties of the molecule showed that all water molecules were removed, hydrogen atoms were added to the protein, all atom force field (OPSL-2001) charges and atom types were assigned. Picture 4 and 5 shows three dimensional of database *NF-kβ.*



Picture 4. Biological Assembly Image for 1NFK – Structure of The Nuclear Factor Kappa-B (*NF-kβ*) p50 Homodimer

Note: Protein chains are colored from N-terminal to the C-terminal using a rainbow (spectral) color gradient [4][17].



Picture 5. Molecular Surface Image 1NFK (*NF-kβ*) with *PyRx* [16]

Docking Simulations

Three flavonoids ligands i.e. cyclomulberrin, cyclomorusin and engeletin and molecule target i.e. *NF-kβ* were simulated with PyRx docking software with AutoDock Vina. AutoDock Vina is a variety of stochastic global optimization approaches were explored, including genetic algorithms, particle swarm optimization, simulated annealing and others, combined with various local optimization procedures and special ”tricks” to speed up the optimization[1].

The docking process used vina search space with center position X:2,0961; Y:13,6606; Z:19,4335 and dimension space X:93,5957 Å; Y:101,7283 Å; Z: 80,8913 Å. Each flavonoid were tested 5 times, and each time give nine result of binding affinity (kcal/mol).

Result and Discussion

Table 1. Result.

Ligands	Binding Affinity				
	Test 1	Test 2	Test 3	Test 4	Test 5
1NFK_Cyclomorusin	-7,9	-8,5	-7,9	-7,9	-7,9
1NFK_Cyclomorusin	-7,7	-8,2	-7,7	-7,7	-7,7
1NFK_Cyclomorusin	-7,6	-8	-7,7	-7,6	-7,7
1NFK_Cyclomorusin	-7,6	-8	-7,6	-7,6	-7,6
1NFK_Cyclomorusin	-7,4	-7,9	-7,5	-7,5	-7,5
1NFK_Cyclomorusin	-7,3	-7,8	-7,5	-7,4	-7,4
1NFK_Cyclomorusin	-7,3	-7,7	-7,4	-7,3	-7,3
1NFK_Cyclomorusin	-7,3	-7,6	-7,3	-7,3	-7,3
1NFK_Cyclomorusin	-7,2	-7,6	-7,3	-7,3	-7,2
1NFK_Cyclomulberrin	-7,3	-7,4	-7,4	-7,4	-7,4
1NFK_Cyclomulberrin	-7,3	-7,4	-7,3	-7,3	-7,1
1NFK_Cyclomulberrin	-7,1	-7,1	-7,2	-7,3	-7
1NFK_Cyclomulberrin	-7,1	-6,9	-7,1	-7,2	-6,9
1NFK_Cyclomulberrin	-7	-6,9	-7	-7	-6,9
1NFK_Cyclomulberrin	-7	-6,9	-6,9	-6,9	-6,9

1NFK_Cyclomulberrin	-6,9	-6,7	-6,8	-6,8	-6,8
1NFK_Cyclomulberrin	-6,9	-6,7	-6,7	-6,7	-6,7
1NFK_Cyclomulberrin	-6,8	-6,6	-6,7	-6,7	-6,7
1NFK_Engeletin	-9,5	-9,5	-8,9	-9,5	-9,4
1NFK_Engeletin	-9,4	-9,4	-8,9	-9,3	-9
1NFK_Engeletin	-9,3	-9,3	-8,8	-9,1	-8,9
1NFK_Engeletin	-9,2	-9,2	-8,8	-8,9	-8,9
1NFK_Engeletin	-9,1	-9,1	-8,8	-8,8	-8,9
1NFK_Engeletin	-9	-8,9	-8,6	-8,8	-8,8
1NFK_Engeletin	-8,9	-8,9	-8,6	-8,8	-8,8
1NFK_Engeletin	-8,8	-8,8	-8,6	-8,7	-8,6
1NFK_Engeletin	-8,8	-8,8	-8,6	-8,7	-8,6

According table I 135 result are shown and all docking process was complete and didn't have problem. Each ligand have 9 reslut in one test and 45 test in all test. We averaged the result each of test. So, we have 5 score of binding affinity average from all test. We total all average for each ligand and we averaged again to get total average of all test. Calculation will shown in table II.

Table II - Binding affinity average of three flavonoids

Ligands	Binding Affinity Average					Total Average
	Test 1	Test 2	Test 3	Test 4	Test 5	
1NFK_Cyclomorusin	-7,48	-7,92	-7,54	-7,51	-7,51	-7,59
1NFK_Cyclomulberrin	-7,04	-6,96	-7,01	-7,03	-6,93	-7,00
1NFK_Engeletin	-9,11	-9,10	-8,73	-8,96	-8,88	-8,96

The best binding affinity from overall average shows engeletin compound followed by cyclomorusin and cyclomulberrin. It shows that engeltin have the ability to interact with *NF-kβ*better than cyclomorusin and cyclomulberrin.bioactivity in particular types of interactions that occur can not be known or inferred because there were so many binding places that occur between ligands and macromolecule (receptors).

Conclusion

Three flavonoids have biochemical interactions with *NF-kβ* virtually in our study. AutoDock Vina proved to be very fast in the process of docking and also excellent in calculation of binding affinity.Engeletin have binding affinity score of –8,96 kcal/mol, followed by cyclomorusin with -7,59 kcal/mol and cyclomulberrin with -7,00 kcal/mol. Those result indicate three flavonoids have ability to interact with *NF-kβ*. We greatly hopes for this research to be developed further so that it could potentially be a ligand as an anti-inflammatory drug, tumor prevention and other benefits that associated with *NF-kβ*.

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