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MOLECULAR DOCKING ANALYSIS OF APIGENIN AS A NATURAL ANTICANCER COMPUND

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ABSTRACT

Objective: Investigation of the anticancer mechanism of apigenin bioflavonoid compounds was carried out through docking experiments with 3 different molecular targets; Vascular Endothelial Growth Factor R2 (VEGFR2), Procaspase 7, Protein Kinase B were known to be involved in the physiology of cancer. The interaction of apigenin was compared with the interaction of each original crystallized ligand at the active location of this receptor. This comparison was based on the parameters of their docking results, as well as, the type of interaction that occured with various amino acids on the active side of the bonding pocket. **Materials and Methods:** Molecular docking studies began by downloading the receptor file as a target on the Protein Data Bank (PDB), and the ligand structure was obtained from pubchem and zinc. docking. The preparation of receptors and ligands was done with discovery studio software, pyrx, MgTool, followed by docking and visualization processes using AutoDock Vina and Discovery Studio Visualizer. **Results:** The binding activity value as a docking score was obtained -7.6 kcal/mol for interaction with the procaspase 7 receptor, protein kinase B receptor B -9.1 kcal/mol, and VEGFR2 -7.3 kcal/mol. **Conclusions:** Molecular docking studies showed that the flavonoid apigenin compound had the strongest affinity and potential as an anticancer through the inhibition mechanism of protein kinase B receptors.

KEYWORDS: molecular docking, anticancer, apigenin, flavonoid, VEGFR2, procaspase 7, protein kinase B.

INTRODUCTION

Cancer is a group of diseases caused by abnormal cell growth with the potential to attack or spread to cells in other parts of the body. Cancer is the second most common cause human death throughout the world.[1] Despite, the progress of research on cancer is growing rapidly, there is still an urgency to find and develop anti-cancer therapy. Natural compounds are still in great demand as chemopreventive agents because of their low levels potential toxicity and efficacy effects.[2]

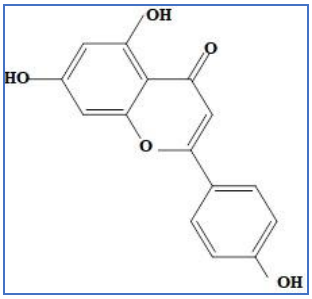

Figure 1: Structure of apigenin.

Apigenin is a natural compound of flavonoids that can be found in several plants including Matricaria chamomilla, onions, parsley, wheat germ, celery (Apium graveolens).[3] Apigenin compounds have the molecular formula $C_{15}H_{10}O_5$ and known chemically as 4 ′, 5,7-trihydroxyflavone has been widely studied as having antioxidant activity through the mechanism of xanthine oxidase inhibition, and interfering with superoxidase activity.[4] In addition, Apigenin has shown extensive anticancer effects including colorectal cancer, breast cancer, liver cancer, lung cancer, melanoma, prostate cancer and osteosarcoma.[5-8]

Conventional drug discovery techniques require a relatively long time, and also large research costs. Thus, the approach of computational chemistry methods to rationally design drugs combined with structure-based modelling and rapid screening methods offer significant potential for identifying and developing lead anticancer molecule.[9] In silico studies have been carried out to explore the interaction of small molecules such as peptides with target receptors, to identify their chemical physical properties.[10]

In this study, three receptor targets related to cancer and cancer cell growth were selected for predicting the potential targets of apigenin. The proteins were procaspase 7 (PDB ID: 1K88), protein kinase B (PKB; PDB ID: 1GZN), and Vascular Endothelial Growth Factor R2's receptor kinase (VEGFR2; PDB ID: 1VR2).

Vascular endothelial growth factor (VEGF) is a crucial factor in the process of angiogenesis.[11] The biological effects of VEGF are mediated by two tyrosine kinase receptors; that is, Flt-1 (VEGFR-1: R1) and KDR / Flk-1 (VEGFR-2: R2), which are very different in signaling properties.[12] VEGF-R2 interactions are the main regulator of antiapoptotic effects and maintenance of sinusoidal endothelial cell (SEC) architecture.[13]

Protein kinase B (PKB) or known Akt plays a central role in the regulation of metabolism, cell survival, motility, transcription and the generation process of the cell cycle.[14] In cancerous conditions, high constitutive PKB activity levels can be caused by various mechanisms, including Pkb/Akt gene amplification and mutations in the PI 3-kinase signaling pathway component.[15]

Activated caspase-3 and caspase-7 can break down large amounts of structural proteins and regulators, which are very important for regulating cell survival and maintenance.[16] Zymogen procaspase-7 is a polypeptide chain structure, with L2 loops connecting large and small subunits. This interdomain loop contains a cleavage site (Ile195-Gln196-Ala197-Asp198) for the acSimilar substitution of Procaspase-7, which requires a certain degree of flexibility in this region to bind to the active site.[17]

MATERIALS AND METHODS

Software and Tools

Protein Data Bank (PDB), PubChem, ChemDraw Ultra 12.0, AutoDock Vina 1.1.2, MGL tools, Discovery Studio Visualizer, Pyrx.

Ligand Preparation

Apigenin and various ligands (positive control) were used as ligands for docking studies were listed in Table 1.

Table 1: Ligands used in the study.

No	Ligand	Molecular Formula	References
1	Apigenin	$C_{15}H_{10}O_5$	[8]
2	Cilengitide	$C_{27}H_{40}N_8O_7$	[12]
3	RPRTSSF	$C_{36}H_{59}N_{13}O_{11}$	[14]
4	RGDS	$C_{15}H_{27}N_7O_8$	[16]

Protein Preparation

The target receptor file can be downloaded at Potein Data Bank (http://www.rcsb.org). The proteins used in this study are procaspase 7 (PDB ID: 1K88), protein kinase B (PDB ID: 1GZN), and vascular Endothelial Growth Factor R2's receptor kinase (PDB ID: 1VR2). The file for each receptor in the PDB file format will first be converted to PDBQT file format using the AutoDock tools program.

Docking Studies Using AutoDock Vina

The structure of apigenin and ligand (positive control) which has been minimized energy will bind the target protein using AutoDock Vina 1.1.2. Modified receptor files containing atomic and ligand loads must first be changed from the pdb format to the PDBQT file format using the Open Babel program. The docking process is carried out in receptors inside the box, with a box spacing of 1 Å. This is to keep the receptors rigid and the ligands as interacting molecules will remain flexible. The interaction energy between the ligand and receptor is calculated for all binding sites and is assessed as a binding affinity score (kcal/mol).

Protein–Ligand Interactions. The Discovery studio visualizer program was used to explain interactions between target proteins and ligands. Docking output files are visualized in 2D interactions. Receptor ligand interactions contain informative data from intermolecular interactions, including types of hydrogen bonds, hydrophobic, which bind to the active side of the receptor bonding pocket.

RESULTS AND DISCUSSION

Ligand Preparation

The ligand structure was obtained from PubChem and zinc. docking and converted to pdb format using Open Babel. The physicochemical properties of ligands can be seen in table 2. All ligand structures are then minimized and stored in PDBQT format by the Pyrx program (Figure 2).

Table 2: Physiochemical parameters of ligand.

No	Ligand	Molecular Weight (g/mol)	Hydrogen Bond Donor	Hydrogen Bond Acceptor	XLogP3-AA	Minimize Energy
1	Apigenin	270.24	3	5	1.7	276.21
2	RGDS	433.42	9	10	-7.3	390.39
3	RPRTSSF	849.9	14	14	-7.7	998.90
4	Cilengitide	588.65	7	8	-1	1216.28

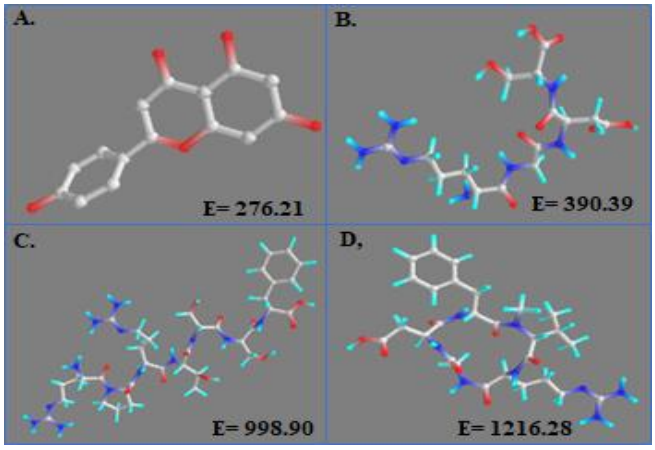

Figure 2: 3D ligand structure and energy minimized results. (A) Apigenin, (B). RGDS, (C) RPRTSSF, (D) Cilengitide.

Protein Preparation

The receptor protein target is downloaded from the Protein Data Bank, the PDB file is converted to PDBQT format using the AutoDock tool (Figure 3).

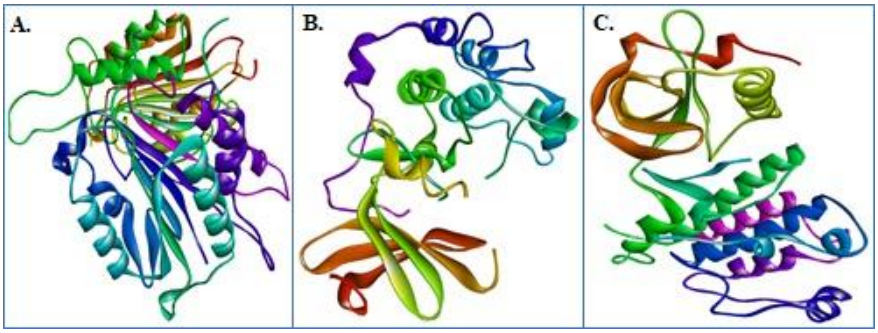

Figure 3: 3D structure of receptor, (A) Procaspase 7, (B) Protein Kinase B, (C) VEGFR2.

Docking Studies Using AutoDock Vina.

Docking of Apigenin into PDB structure of procaspase 7 (PDB ID: 1K88)

Docking simulation results explained that the interaction between RGDS and Apigenin ligands to the procaspase 7 receptor target has many similarities. Interactions on the active side in the receptor binding pocket involve the same amino acids were Val 86, Arg 87, Asn 88, Thr 90, Asp 93, Gly 89, His 144, Arg 187, Gly 238, Ser 233, and Arg 239. Interactions between the RGDS ligand and procaspase receptors have hydrogen bonds on the amino acids Asp 87, Asp 93, Arg 187, Arg 233, and Ser 239. Whereas the ligand apigenin hydrogen bonds with the procaspase 7 receptors occured on the amino acids Arg 87, Asp 93, and His 144.

The docking score obtained can show the interaction between ligand apigenin and Procaspase 7 receptor having a binding affinity score of -7.6 kcal/mol. This result is better than the comparative score between the RGDS ligand with the procaspase 7 receptor of -6.9 kcal /mol. This can explain that Apigenin flavonoid compounds have anticancer activity through inhibition mechanism at Procaspase 7 receptors.

Docking of Apigenin into PDB structure of protein kinase B (PKB; PDB ID: 1GZN)

Interaction diagram results of 2 D ligand-receptors, RPRTSSF ligands and apigenin to PKB receptors have similarities in amino acids Arg 274, Ile 276, Lys 277, Leu 317, Try 334, Gly 335, Val 321, Val 331, Try 334. Ligand RPRTSSF had hydrogen bonds with PKB receptors on the amino acid Thr Lys 181, Val, 198, Thr 199, Ile 276. Whereas the ligand apigenin had a hydrogen bond with the PKB receptor on the amino acid Pro 314. Besides that apigenin ligands had pi alkyl bonds on the amino acid Val 848, Ala 866, and Leu 1035, and Pi sigma bonding also occured in Leu 840.

The interaction between apigenin ligands and PKB receptors had a strong binding affinity with a score of -9.1 kcal/mol better than the RPRTSSF ligand value of -6.6 kcal/mol. This can explain that ligand apigenin has anticancer activity which was very potential through the mechanism of PKB receptor inhibition.

Docking of Apigenin into PDB structure of Vascular Endothelial Growth Factor R2's receptor kinase (VEGFR2; PDB ID: 1VR2)

The interaction of the Cilengitide ligand, apigenin to the VEGFR2 receptor showed the interaction of amino acids on the active site in the receptor binding pocket at Val 848 and Lys 868. Hydrogen bonds are formed between the ligand cilengtide with the receptors on the amino acid Lys 868, Glu 845, and Phe 1047. Formation of hydrogen bonds between the apigenin ligand and the VEGFR2 receptor occured with the amino acids Glu 917, and Cys 919.

The binding affinity score produced between apigenin and VEGFR2 receptor of -7.3 kcal/mol was a strong binding activity which was slightly proportional to the value of binding activity between cilingitide and VEGFR2 receptor. Apigenin as a ligand had potential anticancer activity through the inhibitory mechanism of VEGFR2 receptors. Score binding activity of ligands and various receptors can be seen in the table 4.

Table 4: Comparative binding affinity of different ligands with receptors.

No.	Receptor	Ligand	Binding Affinity (kcal/mol)
1	Procaspase 7	RGDS Apigenin	-6.9 -7.6
2	Protein Kinase B	RPRTSSF Apigenin	-6.6 -9.1
3	Vascular Endothelial Growth Factor R 2	Cilengitide Apigenin	-8.2 -7.3

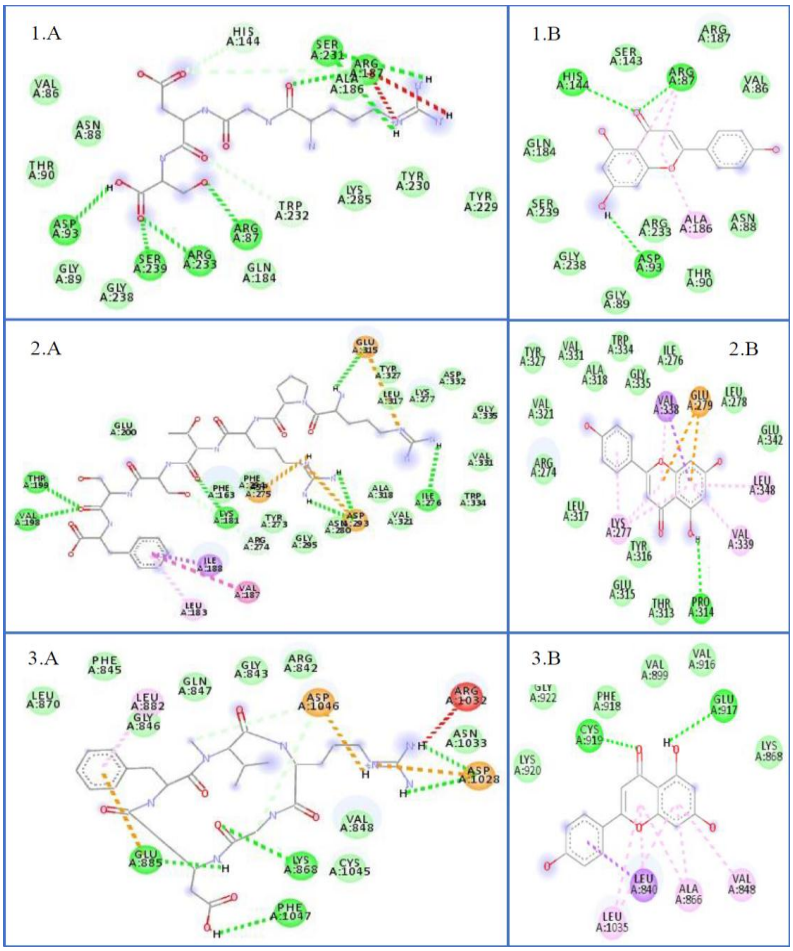

Figure 4: Interaction of ligands and target receptors. (1.A) RGDS bound to Procaspase 7, (1.B) Apigenin bound to Procaspase 7, (2.A) RPRTSSF bound to Protein Kinase B, (2.B) Apigenin bound to Protein Kinase B, (3.A) Cilengitide bound to VEGFR2, (3.B) Apigenin bound to VEGFR2.

CONCLUSIONS

Molecular docking studies of apigenin compounds have the most potential affinity and activity as anticancer agents through the mechanism of inhibiting protein kinase B receptors.

REFERENCES

1. Spaans JN, Goss GD. Drug resistance to molecular targeted therapy and its consequences for treatment decisions in non-small-cell lung cancer. Front Oncol, 2014; 4: 190.
2. Crowell, J.A. The chemopreventive agent development research program in the Division of Cancer Prevention of the US National Cancer Institute: An overview. Eur. J. Cancer, 2005; 41: 1889–1910.
3. Shukla, S dan Gupta, S. Apigenin: A Promising Molecule for Cancer Prevention. *Pharm Res*., 2010; 27(6): 962-978.
4. Xu M, Wang S, Song YU, Yao J, Huang K, Zhu X. Apigenin suppresses colorectal cancer cell proliferation, migration and invasion via inhibition of the Wnt/beta-catenin signaling pathway. Oncol Lett., 2016; 11: 3075–80.
5. Huang C, Wei YX, Shen MC, Tu YH, Wang CC, Huang HC. Chrysin, abundant in Morinda citrifolia fruit water-EtOAc extracts, combined with apigenin synergistically induced apoptosis and inhibited migration in human breast and liver cancer cells. J Agric Food Chem, 2016; 64: 4235–45.
6. Lee YM, Lee G, Oh TI, Kim BM, Shim DW, Lee KH, Kim YJ, Lim BO, Lim JH. Inhibition of glutamine utilization sensitizes lung cancer cells to apigenin-induced apoptosis resulting from metabolic and oxidative stress. Int J Oncol, 2016; 48: 399–408.
7. Zhao G, Han X, Cheng W, Ni J, Zhang Y, Lin J, Song Z. Apigenin inhibits proliferation and invasion, and induces apoptosis and cell cycle arrest in human melanoma cells. Oncol Rep., 2017; 37: 2277–85.
8. Gupta S, Afaq F, Mukhtar H. Involvement of nuclear factor-kappa B, Bax and Bcl-2 in induction of cell cycle arrest and apoptosis by apigenin in human prostate carcinoma cells. Oncogene, 2002; 21: 3727–38.
9. DiMasi, J.A.; Hansen, R.W.; Grabowski, H.G. The price of innovation: New estimates of drug development costs, J. Health Econ, 2002; 22: 151–185.
10. Dileep, K.V.; Kelly, M.; Hardin, E. Approaches in the Chemoprevention of Breast Cancer. Cancer Sci., 2013; 5: 1948–5956.
11. Ribatti D: The crucial role of vascular permeability factor/vascular endothelial growth factor in angiogenesis: a historical review. Br J Haematol, 2005; 128(3): 303-309.
12. Shibuya M: Structure and function of VEGF/VEGF-receptor system involved in angiogenesis. Cell Struct Funct, 2001; 26(1): 25-35.
13. Xia L, Fu GS, Yang JX, Zhang FR, Wang XX: Endothelial progenitor cells may inhibit apoptosis of pulmonary microvascular endothelial cells: new insights into cell therapy for pulmonary arterial hypertension. Cytotherapy, 2009; 11(4): 492-502.
14. Brazil, D. P., Park, J. and Hemmings, B. A. PKB binding proteins. Getting in on the Akt. Cell, 2002; 111: 293-303.
15. Luo, J., Manning, B. D. and Cantley, L. C. Targeting the PI3K-Akt pathway in human cancer: rationale and promise. *Cancer Cell*, 2003; 4: 257-262.
16. Chai, J., Shiozaki, E., Srinivasula, S.M., Wu, Q., Datta, P., Alnemri, E.S., and Shi, Y. Structural basis of caspase-7 inhibiton by XIAP. Cell, 2001; *104*: 769–780.
17. Wei, Y., Fox, T., Chambers, S.P., Sintchak, J.-A., Coll, J.T., Golec, J.M.C., Swenson, L., Wilson, K.P., and Charifson, P.S. The structures of caspases-1, -3, -7 and -8 reveal the basis for substrate and inhibitor selectivity. Chem. Biol., 2000; *7*: 423–432.