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In Silico Molecular Docking Study of Gallic Acid and its Derivatives as Inhibitor BRAF Colon Cancer

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Abstract : Gallic acid is a phenolic acid compound that can be found in natural products and has been reported to have various biological activity against several cancer cell lines such as leukemia, lung cancer, and colon adenocarcinoma. This research is aimed to study the stability, affinity, and interaction of the gallic acid and its five derivatives compounds, namely, ethylgallate, benzylgallate, phenylethyl gallate, (2-hydroxy)-benzylgallate and 4-metoxy-(2-hydroxy)-benzylgallate as inhibitors of BRAF colon cancer by in silico molecular docking. Gallic acid and the five derivatives as a ligand were transformed into 3D structures, subsequently docking simulation process is performed against BRAF. In silico docking study showed the five derivatives have the Gibbs energy (ΔG) value lower than gallic acid, suggesting that the five derivatives have higher stability than gallic acid. Furthermore, compared to gallic acid, the five derivatives have agreater affinity and stronger interaction with the catalytic site of BRAF colon cancer. Among the five derivatives, (2-hydroxy)-benzylgallate has the highest stability and strongest interaction on BRAF colon cancer. Thus, (2-hydroxy)-benzylgallate could be developed as a potential inhibitor of BRAF and promising candidate for colon cancer drug.
Keywords : In silico, Gallic acid, Gallic acid derivatives, BRAF, colon cancer.

Introduction

Gallic acid is a phenylpropanoidcompound[1] that can be found in various natural products[2], and has been reported to have various biological activity toward several cell lines such as leukemia, lung cancer, and colon, as well as in normal lymphocyte cells[3,4,5,6]. Gallic acid very well absorbed in humans, in fact themicromolar concentration of free and glucuronidated forms of gallic acid and has reported major metabolite is 4-O-methylgallate[7,8].

The studies show that the anticancer activity of GA is related to the induction of apoptosis through different mechanisms like generation of reactive oxygen species (ROS), suppression and promotion of oncogenes, regulation of apoptotic and anti-apoptotic proteins, inhibition of matrix metalloproteinases (MMPs) and cell cycle arrest depending upon the type of cancer investigated[9]. Several types of research have shown that gallic acid has the ability to inhibit the proliferation of colon cancer cells to apoptosis. It was reported that the gallic acid can inhibit proliferation and induce apoptosisof cell line Caco-2 colon cancer through activation of

caspase 3[10,11]. Whereas Hwang *et al*, (2007) suggested that Gallic acid is able to inhibit and decrease the expression of COX-2 in HT-29 cells through modulation of AMPK (AMP-activated protein kinase)[12].FurthermoreSubramanian *et al*, (2016) also found that the gallic acid has antiproliferative effect by showing morphological and biochemical changes to apoptosis in cell line HCT-15 colon cancer[13].

The research of interaction molecular gallic acid to protein targets have been conducted by several researchers with the method of molecular docking, including research conducted by Yang *et al*, (2006) showed that the gallic acid has potential as a drug candidate for the treatment of breast cancer and liver by inhibition the activity of epidermal growth factor receptor (EGFR) tyrosine kinase[14]. Gallic acid derivatives, namely aril-3,4,5-trimethyl gallate also has potential as an anti-inflammatory by inhibition the activity of COX-1 and COX-2[15,16].While Amaravani *et al*, (2012) showed that the results of the analysis docking for gallic acid derivatives, that is 2 - [(2E, 4E) -hexa-2,4-dienyl] -3,4,5-trihydroxybenzoic acid, (3.4, 5-trihydroxybenzoyl) 3,4,5-trihydroxybenzoate and 3-hydroxy-4-sulfooxybenzoic as strong inhibitors of the COX-2[17].

One effort that can be done is by inhibition the activity of BRAF mutation. BRAF mutations causing the activation of mitogen-activated protein kinase pathway and EGFR, which plays an important role in cell proliferation and cancer progression[18,19, 20]. It has been reported that BRAF mutations occur about 10% -18% of colon cancer[21,22].This research was designed to know the potential of gallic acid and its derivatives as BRAF inhibitor of colon cancer by observing the affinity and interaction of the ligand when bound to the residue BRAF (5C9C) through the process of molecular docking.

Experimental

Material

The structure of Gallic acid ligand (Fig. 1) and its derivatives as well as comparative ligand (anticancer drugs) (Fig. 3) drawn by the software. While the BRAF crystal structure with code 5C9C downloaded from the Protein Data Bank (http://www.rscb.org/pdb/) (Fig. 2). The tools used are the hardware and software. Hardware in the computer form with the appropriate specifications for the docking process, and software consisting of MarvinSketch 15.5.11, Chimera 1.10.2, Python Molecule Viewer 1.5.6rc3, Autodock 4.2, PyMOL 1.7.4.5 and LigPlot v.1.4.5.

Methods

The first stage is the preparation of macromolecular structures targets include search and downloads, optimization and separation of residues nonstandard.Macromolecular structure downloaded from the web PDB with code 5C9C for BRAF.Further optimization is done by using UCSF Chimera. The second stage is the preparation of the ligand which includes the manufacture of 2D structures, changes in the structure into 3D, as well as the addition of a hydrogen atom and Gasteiger energy. The third stage is done is file creation Grid Parameter File (GPF) and Docking Parameter File (DPF) macromolecular complexes with ligands using Autodock 4.2 software. Furthermore, the process of docking simulations between macromolecular and ligands of operated using the program "command prompt" produces a file with a file format * .glg for GPF and * .dlg to file DPF. The last stage is the analysis of simulation results docking.

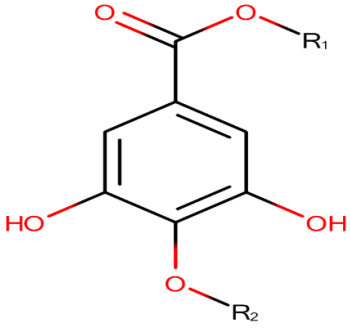


Fig 1. Molecule structure of gallic acid

Fig 2. Crystal structure BRAF

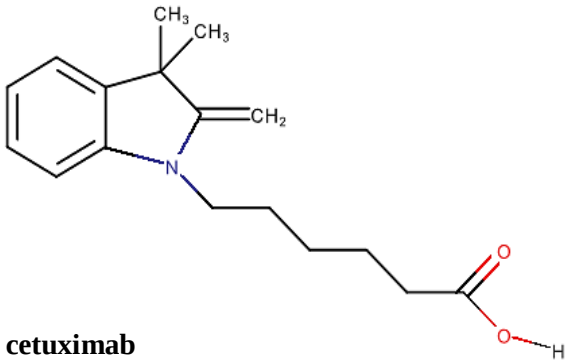


Fig 3. Molecule structure of cetuximab

Result and Discussion

Docking studies were performed to evaluate the effect of ligands on the macromolecules BRAF. The Gibbs energy reflects the interaction energy between the ligand-protein complex and which has the lowest energy showed more stable interactions. Docking simulation results can be seen in Fig. 4.

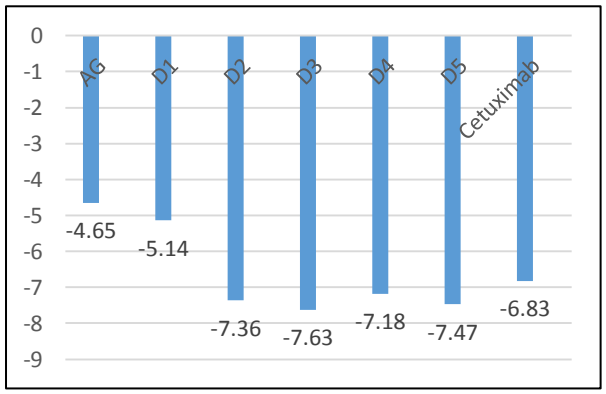


Fig 4. The value of Gibbs Energy (kcal/mol) gallic Acid, its derivatives, and cetuximab

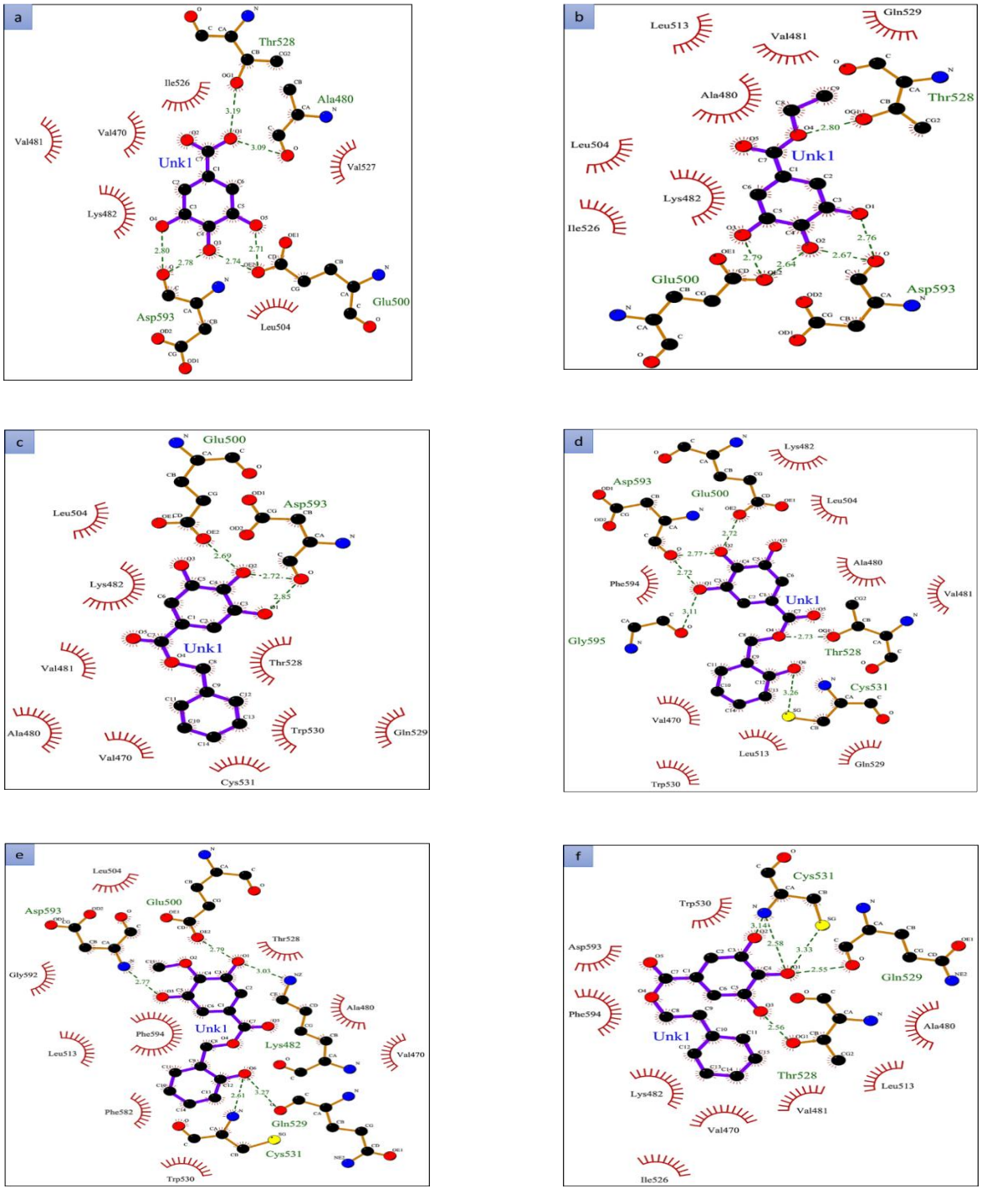
Docking simulation data above indicate that five gallic acid derivatives are ethyl gallate (D1), benzyl gallate(D2), (2-hydroxy) benzyl gallate (D3), 4-methoxy-(2-hydroxy) benzyl gallate (D4) and phenylethylgallate (D5) has good potential on the inhibition of BRAF as an anti-proliferation and induction of apoptosis.Derivative compounds which have the greatest potential as a BRAF inhibitor is (2-hydroxy) benzyl gallate (D3). ΔG Additionally, other indicators docking simulation results showing the standard inhibitor between ligand-protein complex is the inhibition constants (Ki) and the number of hydrogen bonds can be seen in Table 1.

Table 1. Results of the simulation test ligand docking and comparative ligand in BRAF

Comp	R_1	R_2	Ki (μM)	∑IH
GA	H	H	389,42	3
D1	C_2H_5	-	170,87	2
D2	C_7H_7	-	4,03	3
D3	C_7H_7O	-	2,57	4
D4	C_7H_7O	CH_3	5,54	4
D5	C_8H_9	-	3,81	3
Cetuximab	-	-	9,89	1

GA :gallic acid; D1 : ethyl gallate; D2 : 4-methoxy-(2-hidroksi) benzyl gallate; D3 : benzyl gallate; D4 : phenylethylgallate; D5 : (2-hydroxy) benzyl gallate; Ki (inhibition constant); ∑IH (amount of hydrogen bond); Rmsd (Root mean square deviation).

Docking simulations good indicator can be seen by comparing the value of the Gibbs energy (ΔG), inhibition constant, and the amount of hydrogen interaction as astandard inhibitor.A bond forming strong complexesis characterized by a low ΔG value, lower inhibition constants, and the large number hydrogen interactions.Based on the simulation results of docking four gallic acid derivatives, namely D2, D3, D4, and D5 have a pretty good indicator criteria (based on Fig. 4 and Table 1) and can potentially be used as a colon cancer drug candidates to inhibit the BRAF residue.Compounds that show a strong bond between the ligand-protein complex under the three indicators are derivatives 3 with ΔG value, Ki and ΣIH respectively is -7.63 kcal/mol, 2.57 μM, and 4.



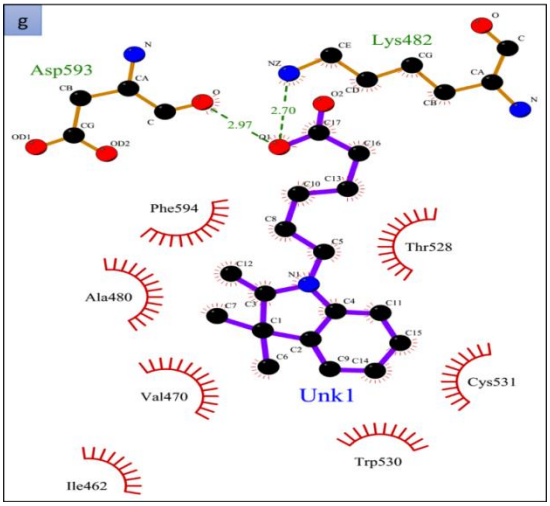


Fig 5. Interactions of BRAF residue with gallic acid and its derivatives, as well as comparative ligand; a) gallic acid, (b) ethyl gallate, (c) benzyl gallate, (d) 2-hydroxy benzyl gallate, (e) 4-methoxy (2-hydroxy) benzyl gallate, (f) phenylethylgallate, and (g) cetuximab

Bonds between the ligands with the BRAF residue was hydrogen bonding which binds to amino acids LYS^{482}, GLU^{500}, LEU^{513}, THR^{528}, GLN^{529}, CYS^{531} and ASP^{593} distances <3,5 Å (Fig. 5).Most of the interactions that occur are the amino acids GLU^{500} and ASP^{593} that show stability and a very solid bond between ligands (gallic acid and its derivatives) with residues.Whereas for the comparison ligand is cetuximab give weak hydrogen bonds to LYS^{482}.Research conducted Yang *et al* (2011) demonstrated that amino acids Glu^{501}, Cys^{532}, and Asp^{594} with a distance <3.5 Å give a very strong hydrogen bonds and stable in BRAF residue[23].

Conclusion

The study results molecular dockingshowed that the four derivatives of gallic acid are D2, D3, D4, and D5 potential as a colon cancer drug candidate compared with gallic acid and an anticancer drug cetuximab is based on three indicators emphasize that ΔG, Ki and ΣIH.Docking simulations reflecting the initial step in the development of the discovery of new drug candidates.Furthermore, the research needs to be done by invitro / in vivo to assess the potential compound gallic acid and its derivatives as a drug candidate in the treatment of colon cancer.

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