International Journal of ChemTech Research
CODEN (USA): IJCRGG, ISSN: 0974-4290, ISSN(Online):2455-9555
Vol.10 No.3, pp 111-117, 2017

In Silico Study of Gallic Acid Derivatives as Novel Antiviral Agents of Hepatitis C

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Abstract: In this paper, we report in silico study of gallic acid derivativesas novel antihepatitis C virus agents. The derivatives were designed by expanding the carboxyl group of gallic acid with open-chain moiety of L-threonine-allyl esters, as well as to modify the hydroxy groups on the aromatic ring of gallic acid with methoxy group in the derivatives. Designed compounds and the original gallic acid were docked based on their interaction with hepatitis C virus receptor binding target NS5B. Compared to gallic acid, all the twenty designed compounds, exhibited higher binding energy, affinity, and hydrogen bond interaction on receptor target of NS5B, indicating that the designed compounds have a stronger inhibitory activity against NS5B.
Keywords : In silico docking, gallic acid, stereocentre derivative, antiviral, Hepatitis C.

Introduction

Hepatitis C Virus (HCV) is one of the main pathogens to cause chronic hepatitis, cirrhosis and hepatocellular carcinoma (HCC). HCV has infected 2.8% the world population (about 180 million individuals according to the database of World Health Organization), and 3-4 million are newly infected each year. Progression of HCV was slow and had mild symptoms. It will make a stealth epidemic and most infections progress a chronic state that persists for decades. People that infected by HCV about 60%-80% will develop chronic hepatitis, of which about 20% develop cirrhosis, and approximately 2%-5% of patients died of liver cirrhosis and liver cancer. More than 350 thousand people die every year from hepatitis related liver diseases by HCV infection, for example, cirrhosis, liver failure, and HCC[1].

There are an estimated 130–150 million people living with chronic hepatitis C virus (HCV) infection worldwide. These data indicate the current burden of HCV infection but provide limited insight into temporal trends in new infections[2]. The prevalence of HCV infection varies worldwide. Including endemic areas is quite high among Southeast Asia, including Indonesia, the number of patients with 7 million people[3]. Various therapies have been conducted for the treatment of hepatitis C virus infections, including the use of combination therapy with interferon alpha or pegylated interferon as an immunomodulator, with ribavirin, boceprevir or telaprevir as an antiviral nucleoside analogue, which is effective to inhibit the growth of cells of hepatitis C[4-6]. However, recent research reveals that antiviral treatment of hepatitis C therapy that is used today, in addition to

poor tolerance to some patients and severe side effects, also has a high resistance level, so it is not effective longer used[7]. This fact encourages the efforts to continue to conduct research and development of hepatitis C antiviral drugs that are more effective and saver.

Gallic acid (GA; 3,4,5-trihydroxyl-benzoic acid) is compound which is widely distributed in various plants, fruits, and foods. Gallic acid was demonstrated to have various biological activities including antibacterial, antiviral, and anti-inflammatory[8]. In 2005, researchers from China revealed that the gallic acid (**1**) is contained in the ethanol extract of Chinese herbal plant *Saxifraga melanocentra*, showed antiviral activity by inhibiting the activity of HCV NS3 serine protease[9-10]. Similar with these results, Sharaf and co-workers in 2012, reported that the gallic acid (**1**) which is the main component of grape seed extract (*Vitis vinifera* L) shows the inhibitory effect on cell growth of the hepatitis C virus human hepatoma HepG2[11]. GA treatment was found to diminish the cellular oxidative stress by decreasing ROS production, which in turn was unfavorable for HCV. Thus, GA is suggested to be a promising adjuvant in HCV therapy[12].The results of previous studies indicate that the gallic acid is a naturallyobtained compound that potential to be developed as antiviral of hepatitis C. Previous researchers reported that the ester of gallic acid and D-glucose (gallated-D-Glucose ester) is isolated from the Chinese herb *Saxifraga melanocentra*, has several chiral centers of the monosaccharides D-glucose group showing the antiviral activity of hepatitis C is 10 times more powerful than the gallic acid or alkyl esters error which has no chiral center[13]**.**

Meanwhile, based on our research, synthetically modified chemical structure by addition of chiral center (stereocentre) on a derivative compound antimycin A_3has proven to increase their activity as antiviral hepatitis C. Accordingly, the results of this study indicate that the chiral center plays an important role and contribute to increasing in antiviral activity of hepatitis C. Thus, in this study we aim to design and study about molecular docking of gallic acid derivatives with chiral center on compound **10 - 12**(Figure 1). Our previous study also showed that the methylation of the hydroxy group on the aromatic ring will enhance the antiviral activity of hepatitis C derivatives, so in this study, we modify the hydroxy groups on the aromatic ring of benzene into monomethoxy, dimethoxy and trimethoxy group on the target compound **13 - 15**,**16 – 18,** and **19 – 21**, respectively.To study the extent to which the stereochemistry affect the antiviral activity of hepatitis C, so we designed the chiral center at bottom facial stereochemistry (R configuration) at the hydroxyl group of target compounds **11, 14, 17** and **20** (marked with a dotted red line), in contrast, with top facial stereochemistry (S configuration) at the hydroxyl group of target compounds **12, 15, 18** and **21** (marked with thick blue line). Addition of chiral center on open-chain structure of the target compounds gallic acid derivatives can be expected to significantly increase the activity, effectiveness and efficiency as an antiviral agent of hepatitis C.

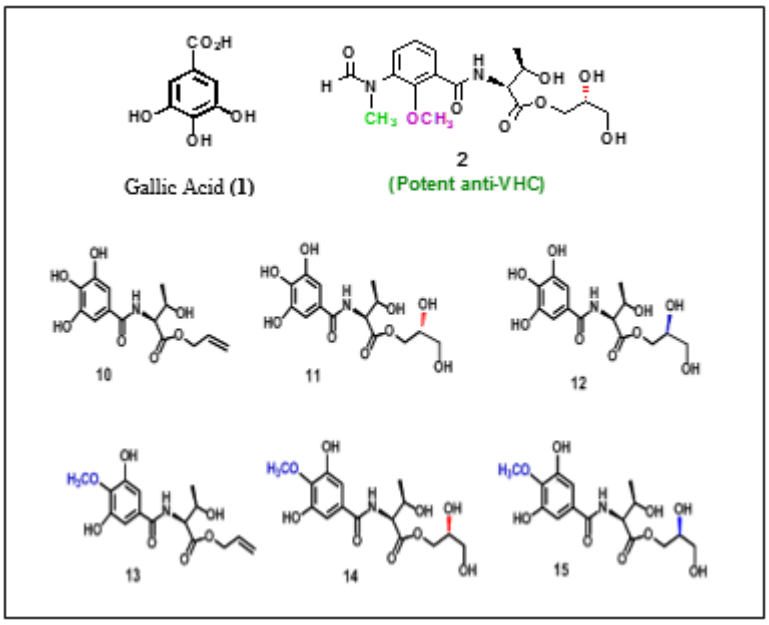

Figure 1. Structure of gallic acid (1), gallic acidderivative (10) - (15)

Methods

In this research, we simulated some derivative compounds of gallic acid based on their interactions with NS5Bhepatitis C cancer, using computer software applications (*Molecular* method)[14](Vidal et al., 2011) to determine the best compounds[15](Wang et al., 2009). Analysis and screening werebased on Gibbs Free energy (ΔG) values, affinity, conformation of the structure, and hydrogen bonding interaction between compounds and thetarget proteins[16](Kruger et al., 2010).

Sequence alignment and homology modelling

Target protein sequences were selected and downloaded from NCBI (http://www.ncbi.mlm.nih.gov/genomes//). The multiple sequence alignment method was based on clustal W2 program (www.ebi.ac.uk/Tools/clustalw2/index.html). Homology modeling was performed using the Swiss Model which can be accessedthrough http://www.swissmodel.expasy.org/SWISS-MODEL.html. Swiss model showed that NS5B has structurallyhomologous to a target protein with template PDBcode 1g5mA (target region 3-204, 88.00 % of sequence identity).

Structural Analysis of Target Protein

Validation of 3D structure from homologymodeling was performed using the Protein Geometry program and superimposed using superpose program in MOE2009.10 software. Based on superimposed the RMSD was calculated to find out structural similarity betweentemplate model mutated with 3D structure fromhomology modeling. Identification of catalytic site of protein target using site finder program in MOE 2009.10 software.

Optimization and Minimization of 3D Structure

Optimization and minimization of three-dimensional structure of the enzyme were conducted using the software of MOE 2009.10. with addition of hydrogen atoms. Protonation was employed with protonate 3D programs. Furthermore, partial charges and force field were employed with MMFF94x. Solvation of enzymes was performed in the form of a gas phase with a fixed charge, RMS gradient of 0.05 kcal/A^0mol, and other parameters using the standard in MOE 2009.10 software.

Preparation of Compounds

Some gallic acid derivatives were designed using ACD Labs software. With this software, The analogues were built into three-dimensional structures. The three-dimensional shape was obtained by storing the derivative in the 3D viewer in ACDLabs. Furthermore, the output format was changed into Molfile MDL Mol format using the software Vegazz to confirm for the docking process. Compounds were in the wash with compute program, adjustments were made with the compound partial charge and partial charge optimization using MMFF94xforcefield. The conformation structure energy of compounds was minimized using the RMS gradient energy with 0,001 kcal/A^omol. Other parameters were in accordance with the default setting in the software.

Molecular Docking

The docking process was begun with the docking preparation, that was employed using a docking program from MOE 2009.10 software. Docking simulations were performed with the Compute-Simulation dock program. The placement method was conducted using a triangle matcher with 1.000.000 repetition energy reading for each position and other parameters were in accordance with the default settings in the MOE software. Furthermore, scoring functions used London DG, refinement of the configuration repetition forcefield with 1.000 populations. The first repetition was done for 100 times and the second setting was conducted only for one of the best result.

Results and Discussion

The molecular docking process predicts ligand confirmation and orientation within their targeted binding site which holds great promise in the field of computer-based drug design[17]**.** Twenty designed compounds (**Figure 2**), including thederivatives (**10) - (21)**, open-chain core of threonine-allyl-ester as

ammonium kloride salt (**9**)and simple benzoic acid ring segments (**1) – (7)**, were simulated using molecular docking on target protein of NS5B hepatitis C virus.

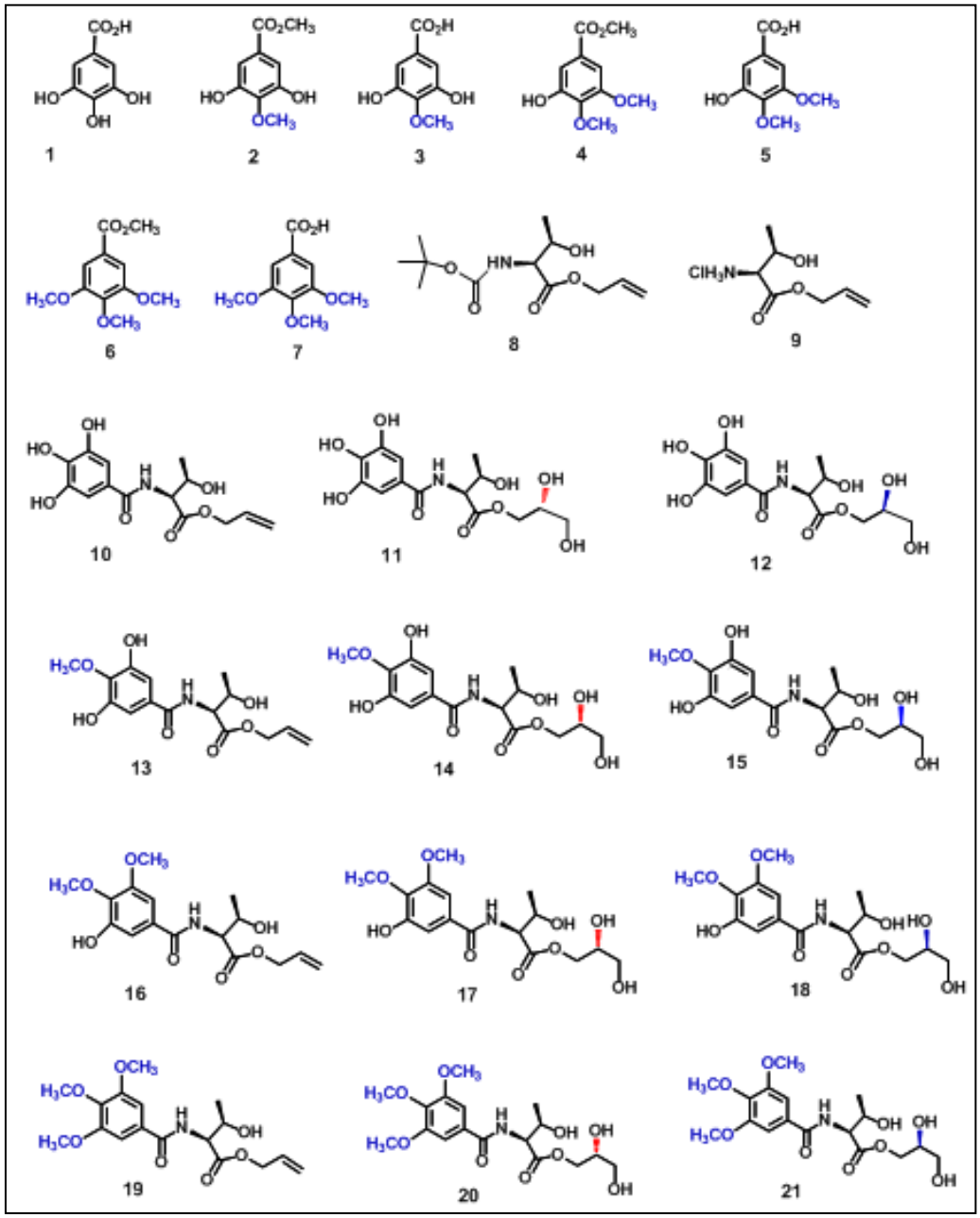

Figure 2. Structure of designed compounds

The results are displayed in Table 1. The top-ranked compounds were selected based on low ΔG binding energy, high pK_i affinity, and number of hydrogen acceptor/ hydrogen donors (hydrogen bonding interaction) to the catalytic site of NS5B target protein.As shown in Table 1, compared to gallic acid, all the twenty designed compounds, exhibited higher binding energy, affinity, and hydrogen bond interaction on receptor target of NS5B, indicating that the designed compounds have a stronger inhibitory activity against NS5B.

Table 1.The Properties of twenty designed compounds and gallic acid (1) on the catalytic site of NS5B

Compound	ΔG (Kcal/mol)	pKi (μM)	Hacceptor/H donor interaction
Gallic acid (**1**)	-5.5931	5.971	3
2	-6.1742	5.301	1
3	-8.3567	5.321	2
4	-7.1244	5.125	1
5	-8.4908	7.675	5
6	-8.4001	6.562	3
7	-7.2450	6.203	2
8	-6.2114	6.123	2
9	-7.0897	6.205	3
10	-10.7254	6.371	1
11	**-11.8169**	**10.751**	**10**
12	**-10.6663**	**7.875**	**5**
13	-8.3558	7.005	3
14	-8.3560	7.106	3
15	-9.6601	7.050	5
16	-8.3450	6.824	2
17	-9.3422	7.379	4
18	-9.5484	8.885	6
19	-8.3330	6.423	3
20	-9.7132	7.118	5
21	-8.5255	7.421	4

The docking of the derivatives compound **10-21**, produced the two top-ranked compounds, namely, compounds **11** and **12**(marked as blue color),which showed lower ΔG binding energy valueand a higher number of hydrogen bonding interaction than the others compounds. The ΔG valuesof compounds **11,** and **12** are-11.8169and -10.6663 kcal/mol, respectively, which are better than gallic acid (**1**), with a ΔG value of -5.5931kcal/mol.These results showed that, compared to gallic acid, those two top-ranked compounds will form a more stable complex with NS5B, as well as, be better able to inhibit and reduce the activity of NS5B. The pKi value of the two top-ranked compounds are higher than gallic acid, indicating that they have a higher affinity and interact effectively with the target NS5B. Moreover, all of those two top-ranked compounds have a number of hydrogen acceptor/hydrogen donor interactions more than gallic acid, which demonstrated greater inhibitory activities on receptor target NS5B.These favored ligand modes were stabilized by hydrogen bonds between the functional group from the ligands with the functional group of side chain residues of caspase protein.

If a compound interacts with the catalytic site of the protein target, it will reduce the activity of the target protein,and change the protein conformation. Generally, the interaction of the compound with the complex protein target is the hydrogen bond (figure 4). The quantities of hydrogen bond interactions of the compoundwiththe catalytic site of the target protein indicate its ability to inhibit the protein target. Figure 3displays the ligand complex interaction of the two top-ranked compounds (**11**and **12**) with the receptor target NS5B.

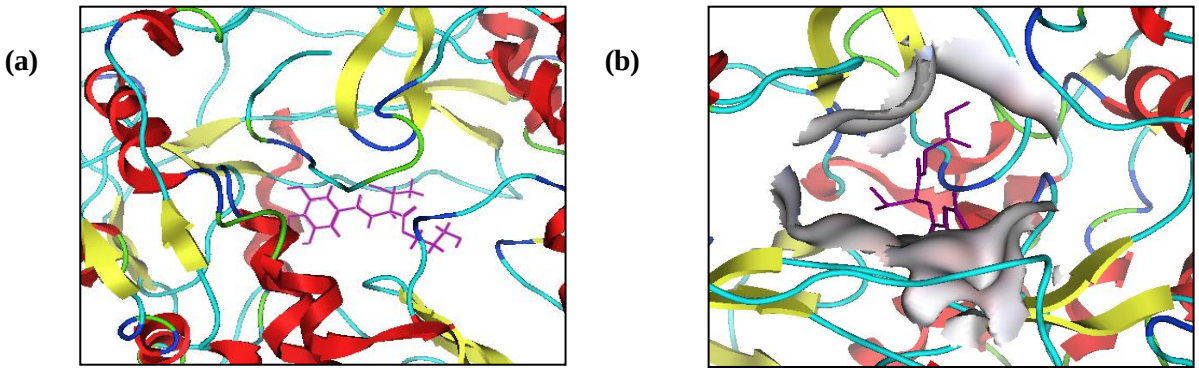

Figure 3. Interaction of compound 11 (a) and 12 (b) on the catalytic site of NS5B.

As shown, all the two of top-ranked compounds could change the conformation of the receptor target cavity, and were able to enter the binding site of the receptor target NS5B. In addition, compared to gallic acid, derivative compounds showed more hydrogen binding interaction against NS5B. The docking results revealed **11** which bears hydroxylated open chain core with bottom facial stereochemistry of chiral center, has more binding interaction, a more stable conformation and a stronger inhibitory activity on the catalytic site of NS5B than gallic acid. Similar to **11**, compound **12**bearingchiral center at the top facial stereochemistry on open chain core as a ligand, also showed stable conformation and strongly inhibited the activity of the NS5B catalytic site.

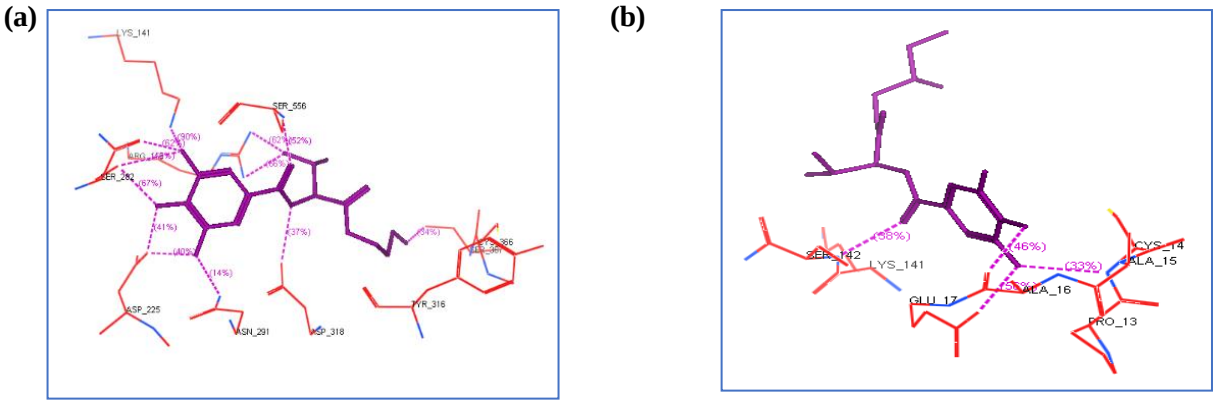

Figure 4.hydrogen binding interaction of compound 11 (a) and 12 (b) against NS5B

These docking results confirmed that introducing bottom facial stereochemistry of chiral center on the hydroxylated open chain corein compound **11** and **12** could remarkably improve its inhibitory activity against the receptor target NS5B of hepatitis C virus.Inconsistent with our previous study, compounds that have methoxy group on the aromatic ring,didnot have better antiviral activity of hepatitis C than compounds without methoxy group. Based on the same top facial stereochemistry on open chain core, i.e compounds **12, 15, 18** and **21,** the order of ΔG values was follow the number of methoxy group, with the ΔG values of compound **12** that did not have the methoxy group, was highest than another. The methoxy group will make compouds too lipofilic and may decrease antiviral activity. Thus, compound **11** and **12**arepromising candidates for new agents of anti-hepatitis C virus.

Conclusion

In conclusion, we have simulated twenty designed compounds by molecular docking approach. Among them, the derivative **11**which have bottom facial stereochemistry of chiral center on the hydroxylated open chain core, demonstrated stronger inhibitory activity and greater interaction on the catalytic site of NS5B hepatitis C virus, compared to the original gallic acid.

Conflict Of Interest

The Authors declare there is no conflict of interest on this article

Acknowledgements

We wish to express our gratitute to Directorate of Research and Public Service University of Indonesia for the Research Grant, and to the Graduate School of Materials Science, Nara Institute of Science and Technology (NAIST), Japan,for International Research Collaboration Program (NAIST Global Initiative Program).

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