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Research Article

IN SILICO DOCKING STUDIES OF GALLIC ACID STRUCTURAL ANALOGS AS BCL-XL INHIBITOR IN CANCER

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ABSTRACT

Objective: Apoptosis, or programed cell death, forms an important part of the cellular regulation machinery. The Bcl-2 protein family, comprising proapoptotic and antiapoptotic members, forms an important part of the cells internal apoptotic pathway. Overexpression of the antiapoptotic members of the family in a number of cancer cell lines renders them immune to apoptosis and the ability to survive under conditions of cellular stress. Inhibition of the antiapoptotic members of the Bcl-2 family like Bcl-XL is, therefore, an interesting target for the development of anticancer therapy.

Methods: The structure of antiapoptotic Bcl-XL receptor (1YSG) was taken from PDB database. The 23-dimensional structure of gallic acid analogs was designed. The Lipinski properties of gallic acid analogs were calculated using molsoft. Docking studies have been carried out through Autodock 4.0 software.

Results: Molecular docking analysis with gallic acid and their structural analogs showed propyl gallate, benzyl gallate, diallyl gallate, phenyl ethyl gallate, and allyl gallate are more interactive and binding strongly than gallic acid at active site of Bcl-XL.

Conclusion: Further these five compounds should be considered as potential candidates for Bcl-XL inhibitor.

Keywords: Apoptosis, Bcl-2, Antiapoptotic Bcl-XL receptor, Gallic acid, Docking studies.

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INTRODUCTION

Apoptosis is an important cellular process that causes death of the damaged cells [1]. Its malfunction can lead to cancer development [2]. There is growing facts that the processes of neoplastic alteration, sequence involve change in the normal apoptotic pathways [3]. Proteins in the Bcl-2 family are central regulators of programed cell death [4], and members that inhibit apoptosis, such as Bcl-XL and Bcl-2, are overexpressed in many cancers and contribute to tumor initiation, progression, and resistance to therapy [5]. Bcl-XL expression correlates with chemoresistance of tumor cell lines [6], and reductions in Bcl-2 increase sensitivity to anticancer drugs [7] and enhance *in vivo* survival [8]. The development of inhibitors of these proteins as potential anticancer therapeutics has been previously explored, but obtaining potent small molecule inhibitors has proved difficult owing to the necessity of targeting a protein–protein interaction.

In the recent years, traditional system of medicine emerged as a potential source to manage the growing rate of chronic, degenerative, environmental, lifestyle, and stress related diseases like cancer [9]. Among the most promising chemopreventive agents, certain natural polyphenols have recently received a great deal of attention because of their demonstrated inhibitory activity against tumorigenesis. In view of their anticancer properties, these compounds also hold great promise as potential chemotherapeutic agents [10].

One of a well-known polyphenol is gallic acid (3,4,5-trihydroxicbenzoic acid) which has selectively cytotoxic activity against a variety of tumor cells [11]. Gallic acid causes induction apoptosis in 3T3-L1 preadipocytes [12] and several other cell lines. There are two pathways by which apoptosis takes place, the mitochondria-dependent (intrinsic pathway) and the death receptor-dependent (extrinsic pathway) [13]. Gallic acid significantly activates Fas, FasL, and p53 proteins [14]. Both the apoptosis pathways are regulated by Bcl-2 family proteins. Bcl-2 family proteins including proapoptotic proteins Bax, Bak, Bad and Bcl-XS and antiapoptotic proteins Bcl-2, Bcl-XL, and Mcl-1. The ratio of expression of Bax/Bcl-2 is a decisive factor in determining apoptosis [13]. Through upregulation of Bax and downregulation of Bcl-2 gallic acid could induce apoptosis in 3T3-L1 preadipocytes [14]. Gallic acid-induced apoptosis in HeLa cells was accompanied by the slight downregulation of Bcl-2 and the upregulation of Bax [15]. Cory and Adams [16] indicated that mitochondrial release of cytochrome c can be controlled by the Bcl-2 family of proteins. Cells treated with gallic acid showed significantly downregulated Bcl-XL protein and upregulation of Bak and Bad proteins [14].

The objective of gallic acid analogs design is to obtain compounds which more potent and has more specific activity as Bcl-XL inhibitor. Nuclear magnetic resonance structural analysis of the Bcl-XL/Bak BH3 peptide complex showed that the Bak BH3 domain binds to the hydrophobic cleft formed by the BH1, BH2, and BH3 domains of Bcl-XL [17]. It can be concluded that the part of binding pocket of Bcl-XL protein is hydrophobic. Gallic acid analogs designed by methylation and esterification with allyl or alkyl is aimed to make a novel compound which has more hydrophobic characteristic than lead compound. These new analogs are expected to have more hydrophobic interactions with the protein Bcl-XL.

Conventional drug design techniques are based on trial and error testing using cells or animals. High-throughput screening for chemicals with desired bioactivities requires specialized labs that make the process costly. With a growing number of known experimental structures of target molecules, computational methods have been used successfully to supplement and speed up drug discovery. Computer-based molecular

design combines methods of informatics, medicine, and biophysics. This cross-disciplinary field has accelerated drug research by predicting the potential therapeutic effectiveness of designed molecules before laboratories experiments and costly preclinical trials. In addition, computational modeling has led to discoveries of structures of a novel small molecule [18]. In this work, informatics and computational design were used to evaluate gallic acid analogs that could potentially promote the death of cancer cells Apoptosis by inhibition of Bcl-XL protein.

METHODS

Protein preparation

The structure of the antiapoptotic Bcl-XL receptor was taken from PDB (1YSG-pdb id). The ligands and crystallographic water molecules were removed from the protein, and the chemistry of the protein was corrected for missing hydrogen atoms. Following the above steps of presentation, the protein was subjected to energy minimization using Python Molecular Viewer 1.5.6 cr3 [19].

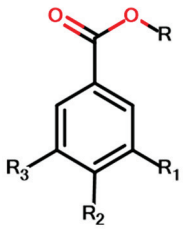
Gallic acid analogs (ligand) structures preparation

The 2D structure of each ligand is displayed as an image generated by MarvinSketch 5.10.0 (ChemAxon) [20]. The 2D chemical structure constructed in the ligand design page by the user is converted to a 3D structure. The 3D ligand structure then undergoes geometry optimization to produce the initial ligand structure. The 2D to 3D transformation, geometry optimization, and molecular format transformation are performed by OpenBabel 2.3.0. The analogs were designed by methylation and esterification of carboxylate groups and hydroxyl groups, respectively. The structures analogs as shown in Table 1.

Drug likeliness evaluation

The drug likeliness of the compounds was evaluated with the help of Lipinski drug filter under Molsoft. This rule describes molecular properties important for a drug's pharmacokinetics in the human body and provides the information regarding the utilization of the ligands as a drug. A promising drug candidate should be "drug-like," which means that it should have characteristics similar to known drugs. Bioavailability, protein affinity, toxicity, transport, absorption, and metabolic stability of a compound depend on many factors. These factors include the compound's hydrophobicity, electronic distribution, hydrogen bonding, molecule size, and flexibility.

Table 1: Design of gallic acid analogs



No	Compound	R	R1	R2	R3
1	Gallic acid	H	OH	OH	OH
2	Propyl gallate	C_3H_7	OH	OH	OH
3	Allyl gallate	C_3H_5	OH	OH	OH
4	Phenyl ethyl gallate	C_8H_9	OH	OH	OH
5	Diallyl gallate	C_6H_{10}	OH	OH	OH
6	4-methoxy propyl gallate	C_3H_7	OH	OCH_3	OH
7	3,4-dimethoxypropyl gallate	C_3H_7	OCH_3	OCH_3	OH
8	3,4,5-trimethoxypropyl gallate	C_3H_7	OCH_3	OCH_3	OCH_3
9	4-methoxyallyl gallate	C_3H_5	OH	OCH_3	OH
10	3,4-dimethoxy allyl gallate	C_3H_5	OCH_3	OCH_3	OH
11	3,4,5-trimethoxy allyl gallate	C_3H_5	OCH_3	OCH_3	OCH_3
12	4-methoxyphenyl ethyl gallate	C_8H_9	OH	OCH_3	OH
13	3,4-dimethoxyphenyl ethyl gallate	C_8H_9	OCH_3	OCH_3	OH
14	3,4,5-trimethoxyphenyl ethyl gallate	C_8H_9	OCH_3	OCH_3	OCH_3
15	Methyl gallate	CH_3	OH	OH	OH
16	4-methoxy methyl gallate	CH_3	OH	OCH_3	OH
17	3-4-dimethoxy methyl gallate	CH_3	OCH_3	OCH_3	OH
18	3,4,5-trimethoxy methyl gallate	CH_3	OCH_3	OCH_3	OCH_3
19	4-methoxy gallate	H	OH	OCH_3	OH
20	3,4-dimethoxy gallic acid	H	OCH_3	OCH_3	OH
21	3,4,5-trimethoxy gallic acid	H	OCH_3	OCH_3	OCH_3
22	Benzyl gallate	C_7H_7	OH	OH	OH
23	4-methoxy benzyl gallate	C_7H_7	OH	OCH_3	OH
24	3,4-dimethoxy benzyl gallate	C_7H_7	OCH_3	OH	OH

Molecular docking of complexes Bcl-XL and gallic acid analogs

The preparative protein and ligand coordinates were saved as pdbqt files. The third step is molecular docking. Grid maps of the predefined active site for each atom type present in the ligand are calculated by AutoGrid before docking a ligand to the target Bcl-XL. After assigning Gasteiger partial charges to the atoms in the ligand, the Lamarckian genetic algorithm based molecular docking and calculation of ligand/Bcl-XL dissociation constant are carried out by AutoDock 4.0 Autodock 4.0 software [21]. The docking parameters are set as follows: The ligand translation step is set to 1.0A°, the ligand quaternion and torsion step are both set to 50°, the maximum number of energy evaluations is set to 1.0×10^6, the maximum number of genetic algorithm operations is set to 2.7×10^4, the number of individuals in the population is set to 150, the rate of mutation and crossover are set to 0.02 and 0.8, respectively. The ligands are set to have prepared as a rigid structure. The complex of protein and ligand are saved as output in dlg files, the grid box volume was adjusted to 40×40×40 Å in the x, y and z axes, respectively, for specific docking of complexes and grid-sizes have space up to 1 Å. Other parameters are all set as default. When searching the conformational and orientational spaces of the ligand with rotatable bonds having full flexibility, the structure of the Bcl-XL is kept rigid. For each docking evaluation, 10 independent runs are performed to evaluate different ligand poses, and only the most favorable pose is dumped to the results file. The results of complexes were generated using Ligandscout 15.1 licensed to Arry Yanuar University of Indonesia.

RESULTS AND DISCUSSIONS

Drug likeliness evaluation

According to Lipinski, 2000 poor absorption and permeation are more likely to occur when the molecular weight is over 500, the octanol/water partition coefficient is over 5 the number of hydrogen-bond acceptors (N and O atoms) is more than 10. As per the view of Smith and Lipinski, for this study, out of 23 gallic acid analogs, 5 compounds with best ΔG binding value are satisfying all the five rules evidencing that this five compounds are able to show drug likeliness.

In this study, five compounds have logP values ranging from 1.64 to 2.82 (Table 2).

Docking results

The binding sites cavity on protein Bcl-XL is very spacious and located on the surface. Interaction in this binding sites are mainly hydrophobic and a little polar hydrogen bonding [22]. Ligand docked into binding site cavity of Bcl-XL. The docking energies of all compounds were represented as ΔG binding in kcal/mol (Table 3). Molecular docking provides information on the assessment and analysis. Assessment is the affinity of the interaction between the ligand and protein. The assessment process is estimated as the value of the free energy of binding ΔG binding. ΔG is used to predict the reaction spontaneity. If ΔG increasingly negative with greater value, the interaction of the ligand and the protein will be more constant and ligand conformation that the protein complex formed will be more stable.

In addition about ΔG, docking process also provides information about the orientation and position resulting from a ligand that acts as an inhibitor of the protein. This is evidenced by the shape conformation between ligands and proteins that interact in general through hydrogen bonding, hydrophobic interactions, and van der Waals bonding.

From the docking results, 23 gallic acid analogs screened for this study, five compounds with the best interaction with the BH3 domain of anti-apoptotic Bcl-XL receptor are shown in Table 3.

The results showed that the five docking derived compounds that have a value ΔG smaller and the pKi value greater than gallic acid also have LogP value greater than gallic acid. This is consistent with previous studies showing that hydrophobic interactions play an important role in bonding between the ligand binding site cavity protein Bcl-XL. The site where the binding protein Bcl-XL broad ligand is a molecule made easy entry into it. Conformation best ligand into Bcl-XL cavity showed in Fig. 1.

The docking structure of the best five compounds is bound in a very similar pattern with the binding site of Bcl-XL receptor. The compounds also have similar volume and radius for the active binding site for defining the sphere. The compounds have different poses in the active site according to its number of hydrogen bond interactions. Interestingly, all the compounds have a good hydrogen bond interaction with the antiapoptotic Bcl-XL receptor.

As shown in Fig. 2, complex of gallic acid and the protein has three hydrogen bonds between the carbon of the benzene ring number three and four with the amino acid residues alanine (ALA108A) and glutamate (GLU 133A) Bcl-XL protein. Gallic acid acts as a hydrogen bond donor. Gallic acid ester derivative, propyl gallate has four hydrogen bonds, two hydrogen bonds as donor and the others as an acceptor (a). Amino acid residues involved in hydrogen bonding propyl gallate is GLU104A, Arg104A, Tyr105A. Three aliphatic carbon atoms in propyl provide hydrophobic interactions. In the complex between the benzyl gallate with the protein showed three hydrogen bond donor with GLU100A amino acid residues, and two hydrogen bond acceptors with amino acid residue ARG 104A dan TYR105A (b). Phenyl side chains undergo hydrophobic interactions with amino acid residues PHE195A, PHE101A, ALA97A, TRY199A, and VAL145A. Allyl gallate complex with the protein indicates a hydrogen bond donor interaction with amino acids GLU96A and one hydrogen bond acceptor with amino acids ARG208A (c). Allyl side chains undergo hydrophobic interactions with the amino acids VAL145A, PHE101A, and PHE195A. In the complex between diallyl gallate and protein, the type of interaction less when compared to the allyl gallate. Hydrophobic interactions between side chains on the allyl ester together with the allyl. However, the hydrogen bond on diallyl only one of hydrogen bond acceptor with amino acids

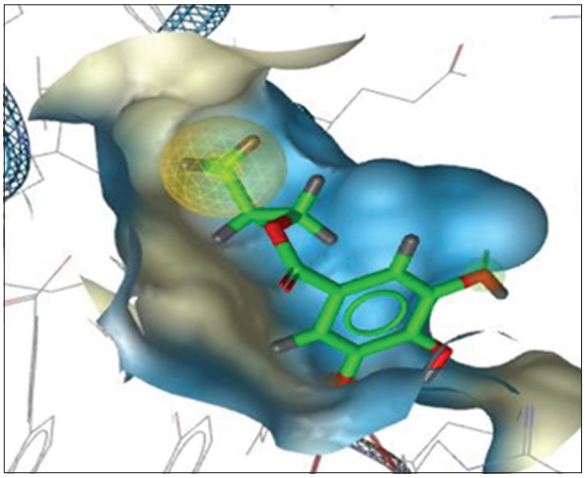


Fig. 1: Complex of best ligand with Bcl-XL protein trough molecular docking analysis

Table 2: Lipinski properties of the five best gallic acid analogs

No	Compound	Molecular weight	HBD	HBA	LogP
1	Gallic acid	170.02	4	5	0.55
2	Propyl gallate	212.07	3	5	1.86
3	Benzyl gallate	246.05	3	5	2.25
4	Allyl gallate	210.05	3	5	1.64
5	Diallyl gallate	250.08	2	5	2.74
6	Phenyl ethyl gallate	274.08	3	5	2.82

HBA: Hydrogen bond acceptors, HBD: Hydrogen bond donors

Table 3: Molecular docking interaction of gallic acid analogs with Bcl-XL

No	Compound	ΔG (Kcal/Mol)	pKi (μM)
1	Propyl gallate	−7.5	1.31
2	Benzyl gallate	−7.3	1.27
3	Diallyl gallate	−7.2	1.25
4	Phenyl ethyl gallate	−6.7	1.17
5	Allyl gallate	−6.5	1.13
6	Gallic acid	−5.5	0.96

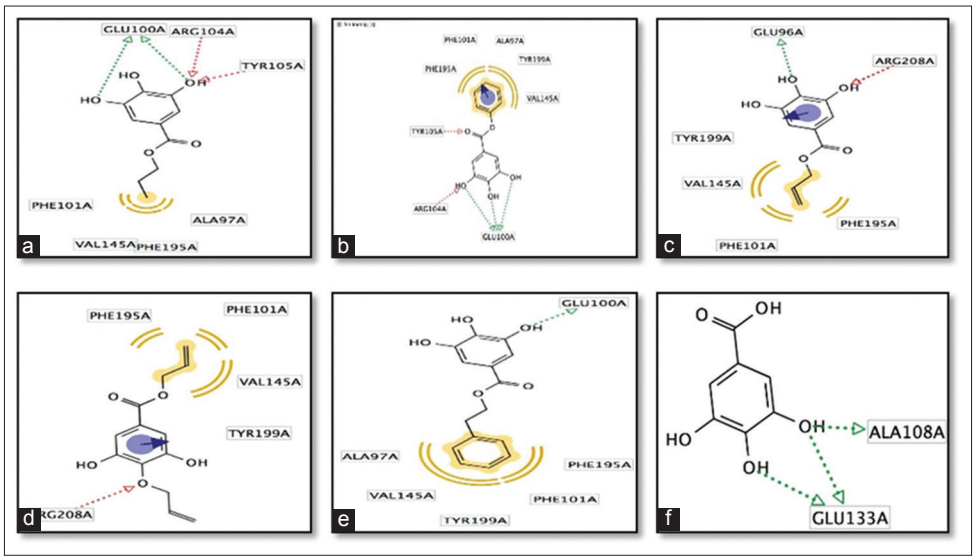


Fig. 2: Interaction of the gallic acid analogs with the Bcl-XL receptor. (a) Propyl gallate, (b) benzyl gallate, (c) allyl gallate, (d) diallyl gallate, (e) phenylethyl gallate, (f) gallic acid. Hydrophobic interaction (yellow), hydrogen bonding acceptor (red), hydrogen bonding donor (green)

ARG208A (d). Complex between ethyl phenyl gallate and protein indicate hydrophobic interactions between side chains of amino acid phenyl with ALA97A, VAL145A, PHE101A, and PHE195A (e). Hydrogen bonding interactions occur only one, that is, with GLU100A as a donor. Based on this data, derivatization of gallic acid provides additional hydrophobic interactions with the protein (f).

The BH3 domain of the proapoptotic protein is thought to be important for the induction of apoptosis. A small molecule interacts with the BH3 binding domain of Bcl-XL/Bcl-2 will function as Bcl-XL/Bcl-2 antagonists and promote apoptosis. In connection to this context in our study the data set of five best compounds inhibiting Bcl-XL receptor also serves to bind to a lesser extent with BH3 binding groove of Bcl-XL receptor. From the insight domain analysis, it is observed that compounds falls often in GLY94, ASP95, GLU96, PHE97, and GLU98 of the BH3 domain of Bcl-XL receptor.

Gallic acid which is polar compound show no hydrophobic interaction at the binding site of Bcl-XL. This is consistent with the expected that decrease polarity will improve the hydrophobic interactions.

CONCLUSION

Protein-ligand interaction studies, in silico from the docking results we conclude five gallic acid analogs have good interaction and inhibitory effect with the BH3 domain of antiapoptotic Bcl-XL receptor better than the lead compound, gallic acid. Hence, we can predict that these five compounds can be an agent for promoting apoptosis. However, this mechanism of prediction requires further *in vitro* and *in vivo* studies for the complete analysis of inducing apoptosis in cancerous cells.

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