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In Silico Docking Studies of Alkyl Esters Derivative of Gallic Acid on Bcl-xL Anti-apoptotic Protein of Breast Cancer

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Abstract : Gallic acid has been reported to have various biological activities including an anticancer effect. Numerous studies have indicated that alkyl esters derivative of gallic acid are more effective as an anticancer against tumor cell lines than gallic acid. Proteins in Bcl-2 family are central regulators of programmed cell death that can inhibit apoptosis, such as Bcl-xl and Bcl-2 which are overexpressed in many tumors. Bcl-Xl permeabilizes the outer mitochondrial membrane of cells and inhibits apoptosis. Most human breast cancers originate from epithelial cells express Bcl-2 or Bcl-xL. Clinical studies have demonstrated that increasing levels of Bcl-xL in breast carcinoma are associated with a poor outcome. In this work, we carried out an in-sukuci study of twenty alkyl ester derivatives of gallic acid (ligands)as an inhibitor of Bcl-xL protein (PDB ID 1YSG) using Autodock 4.2 software. The Gibbs energy (ΔG) showed the stability interaction between ligand and Bcl-xL residues, whereas inhibition concentration (Ki) was used to determine the binding energies of different docking conformation. In silico study showed that among the twenty alkyl esters derivative of gallic acid, five derivative compounds, namely 3,4-dimethoxy-*cis*-2-hexenylgallate; 3,4,5-trimethoxy-*cis*-2-hexenylgallate; 3,4-dimethoxy-*trans*-2-hexenylgallate; 3,4,5-trimethoxy-*trans*-2-hexenylgallate; 3,4,5-trimethoxyhexylgallate have higher stability and stronger inhibitory activity against Bcl-xl than the gallic acid. Moreover, based on in silico results, derivative 3,4,5-trimethoxy-*cis*-2-hexenylgallate with ΔG value of -6.34 kcal/mol and Ki value of 22.47 µM, has the highest potential as an inhibitor of Bcl-xL. This in silicodoking study suggesting that 3,4,5-trimethoxy-*cis*-2-hexenylgallate is a promising candidate to develop as anti-breast cancer agents.
Keywords : In silico docking, gallic acid, alkyl esters derivative, anti-apoptotic, Bcl-xL, breast cancer.

Introduction

Now cancer causes more deaths than coronary heart disease or stroke. Breast cancer is the second most common cancer in the world and the most frequent cancer among women with an estimated 1.67 million new cancer cases diagnosed in 2012 (25% of all cancers). Breast cancer ranks as the fifth cause of death from cancer overall (522,000)[1].A lot of research to synthesis new potential anticancer drugs. Although many potential anticancer drugs have been made but the medical need is still largely unmet due to many factors among which

the lack of selectivity of conventional drugs leading to toxicity, the metastatic spreading, and multi-drug resistance; MDR. A successful anticancer drug should against cancer cells without causing excessive damage to normal cells. This condition is difficult, or perhaps impossible, to attain and is why cancer patients frequently suffer uncomfortable side effects when under-going treatment. Therefore, the search for novel and selective anticancer agents is highly required due to currently available anticancer drugs problems[2,3].

In recent decades, the development of novel and advanced cancer therapiescommon use natural plants, fruits, or foods as a valuable resource. 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, in which the antitumor activity is most striking[3]. Growth of human breast cancer cell, MCF-7, is significantly reduced by treatment with gallic acid. Gallic acid causes apoptosis induction and cells treated with gallic acid showed significantly downregulatedof Bcl-xL protein and upregulation of Bak and Bad proteins[4].

Apoptosis (programmed cell death) is highly conserved and regulated process, which is the primary mechanism for the removal of aged, damaged, and unnecessary cells. Its deregulation can lead to cancer development and poor response to conventional chemotherapy. Cellular proteins of Bcl-2 family are a crucial and fundamental component of apoptosis and include members that either prevent (e.g. Bcl-2 or Bcl-xL) or promote (e.g. Bax or Bak) the membrane permeabilization[5]. Bcl-xL is a member of Bcl-2 family proteins that is located within the mitochondrial membrane. Its pro-survival action is founded on its capacity to prevent Cytochrome C release and the associated caspases activation[7].

Conventional drug designing techniques usually used trial-and-error tests using cell and animal model.With an increasing number of known experimental target molecules, computational methods have been used to significantly supplement and expedite the drug designing process. In silico analysis is the most straight forward approach to discover and predict novel lead molecules in less time and cost. Autodockis good molecular docking software which helps in predicting the binding site of ligand-protein interaction[5,7]**.** In this work, we carried out an in-silico study of twenty alkyl ester derivatives of gallic acid (ligands) as an inhibitor of Bcl-xL protein using Autodock 4.2 software. Bcl-xL represents an attractive target for novel anti-canceragents designed to overcome chemotherapy resistance incancer cells.

Experimental

Drug Likeliness Evaluation

The drug likeliness of the compounds was evaluated with the help of Lipinski drug filter under MarvinView Properties Tools. This rule describes molecular properties important for a drug's pharmacokinetics in the human body and providesthe information regarding the utilization of the ligands as a drug.

Selection and Preparation of Macromolecule

Crystal Structure of the Bcl-xL protein (PDB id: 1YSG)was retrieved from PDB databank (http://www.pdb.org/) (fig. 1). The macromolecule was prepared to calculate the binding energy using Chimera 1.10.2. The polar hydrogens were added whereas the non-polar hydrogens were removed from the protein and the Gasteiger partial charges were further added to the carbon that held the hydrogen. The binding pocket of the protein was determinedby grid based approach using default parameters. The grid maps were generated using docking grids of 34*34*34 points spanning binding pocket of the proteinwith the grid point spacing of 0.375 Å. The grid map coveredthe active site along with the significant portions of the surroundingsurface[6].

Ligand Preparation

The 20 analogs of gallic acid were designed (table 1). The structure of a compound is made in 2D using Marvin Sketch 15.1.19 software and saved in .mol format. The 2D structure of the compound was converted to 3D structure using Chimera 1.10.2. It takes .mol as input and generates 3D structure of the molecule whose optimized ligands can be downloaded in PDB format suitable for Autodock4.2 Tools.

Molecular Docking and their Interaction Studies

Molecular Docking of designed compounds was carried out usingLamarckian genetic algorithm inbuilt in Autodock4.2 toolswith default docking parameters. We selected Autodock4.2tool for the purpose of molecular docking because AutoDock has proven to be an effective tool capable of quickly and accurately predicting bound conformations and binding energies of ligands with macromolecular targets[7].

The success rate in retrieving binding modes of known protein–ligand complexes is an important validation for docking programs. The measure that is usually used to determine whether a binding mode prediction is a success is the RMSD[8]. The RMSD between the lowest energy docked Bcl-xL's ligand pose and the Bcl-xL's ligand native pose was evaluated using PyMol 1.7.4.5.

Docking interactions were clustered to determinethe Gibbs energy (ΔG) and optimal docking energy conformationwas considered as the best-docked pose. The generated conformations had an associated value of the Gibbs energy (ΔG). An estimated inhibition concentration (Ki) was used for determination of binding energies of different docking conformations, ranking in accordance to their binding scores[6].

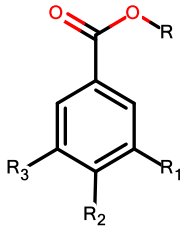


Table 1. Design of Gallic Acid Derivatives

No	Compound	R	R1	R2	R3
1	Gallic acid	H	OH	OH	OH
2	Hexylgallate	C_6H_{13}	OH	OH	OH
3	*trans*-2-hexenylgallate	C_6H_{12}	OH	OH	OH
4	*cis*-2-hexenylgallate	C_6H_{12}	OH	OH	OH
5	3,4,5-trimethoxymethylgallate	C_3H_7	OCH_3	OCH_3	OCH_3
6	3,4-dimethoxymethylgallate	C_3H_7	OCH_3	OCH_3	OH
7	3-methoxymethylgallate	C_3H_7	OH	OCH_3	OH
8	3,4,5-trimethoxygallic acid	OH	OCH_3	OCH_3	OCH_3
9	3,4-dimethoxygallic acid	OH	OCH_3	OCH_3	OH
10	4-methoxygallic acid	OH	OH	OCH_3	OH
11	4-methoxyhexylgallate	C_6H_{13}	OH	OCH_3	OH
12	4-methoxy-t*rans-2*-hexenylgallate	C_6H_{12}	OH	OCH_3	OH
13	4-methoxy-*cis-2*-hexenylgallate	C_6H_{12}	OH	OCH_3	OH
14	3,4-dimethoxyhexylgallate	C_6H_{13}	OCH_3	OCH_3	OH
15	3,4-dimethoxy-t*rans-2*-hexenylgallate	C_6H_{12}	OCH_3	OCH_3	OH
16	3,4-dimethoxy-*cis-2*-hexenylgallate	C_6H_{12}	OCH_3	OCH_3	OH
17	3,4,5-trimethoxyhexylgallate	C_6H_{13}	OCH_3	OCH_3	OCH_3
18	3,4,5-trmethoxy-t*rans-2*-hexenylgallate	C_6H_{12}	OCH_3	OCH_3	OCH_3
19	3,4,5-trmethoxy-*cis-2*-hexenylgallate	C_6H_{12}	OCH_3	OCH_3	OCH_3
20	4-*trans*-2-hexen-oxygallic acid	OH	OH	OC_6H_{12}	OH
21	4-*cis*-2-hexen-oxygallic acid	OH	OH	OC_6H_{12}	OH

Result and Discussion

The drug-likeness of compounds can be predicted by Lipinski's rule of five which refers to the similarity of compounds to oral drugs. 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[8].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, the number of hydrogen-bond donor (N and O atoms) is more than 5[9].In this study, gallic acid and its twenty derivatives are satisfying Lipinski's rules. Five compounds with best ΔG binding value are able to show drug likeliness.

Table2. Lipinski properties of the gallic acid derivatives

No	Compound	Molecular Weight	HBD	HBA	LogP
1	Gallic acid	170.12	4	5	0.72
2	Hexylgallate	254.282	3	4	3.28
3	*trans*-2-hexenylgallate	252.266	3	4	3.07
4	*cis*-2-hexenylgallate	252.266	3	4	3.07
5	3,4,5-trimethoxymethylgallate	226.228	0	4	1.50
6	3,4-dimethoxymethylgallate	212.201	1	4	1.36
7	3-methoxymethylgallate	198.174	2	4	1.21
8	3,4,5-trimethoxygallic acid	212.201	1	5	1.16
9	3,4-dimethoxygallic acid	198.174	2	5	1.01
10	4-methoxygallic acid	184.147	3	5	0.87
11	4-methoxyhexylgallate	268.309	2	4	3.42
12	4-methoxy-t*rans-2*-hexenylgallate	266.293	2	4	3.22
13	4-methoxy-*cis-2*-hexenylgallate	266.293	2	4	3.22
14	3,4-dimethoxyhexylgallate	282.336	1	4	3.57
15	3,4-dimethoxy-t*rans-2*-hexenylgallate	280.320	1	4	3.36
16	3,4-dimethoxy-*cis-2*-hexenylgallate	280.320	1	4	3.36
17	3,4,5-trimethoxyhexylgallate	296.363	0	4	3.72
18	3,4,5-trmethoxy-t*rans-2*-hexenylgallate	294.347	0	4	3.51
19	3,4,5-trmethoxy-*cis-2*-hexenylgallate	294.347	0	4	3.51
20	4-*trans*-2-hexen-oxygallic acid	252.266	3	5	2.87
21	4-*cis*-2-hexen-oxygallic acid	252.266	3	5	2.87

HBD: Hydrogen Bond Donor, HBA : Hydrogen Bond Acceptor

The protein structural topology of the Bcl-xL protein can be described as two hydrophobic helices surrounded by five amphiphilic helices. The binding groove of the Bcl-xL protein is a shallow, solvent exposed, and primarily hydrophobic cavity. It is formed by the side chains originating from the BH (Bcl-2 homology) 1, BH2, and the BH3 domains of the protein. Although the binding groove of Bcl-xL is primarily hydrophobic, there are polar residues forming the walls of the binding pocket. While most of the residues point into the solvent, two residues Arg143 and Glu100 are accessible for interaction with the binding ligand[10].

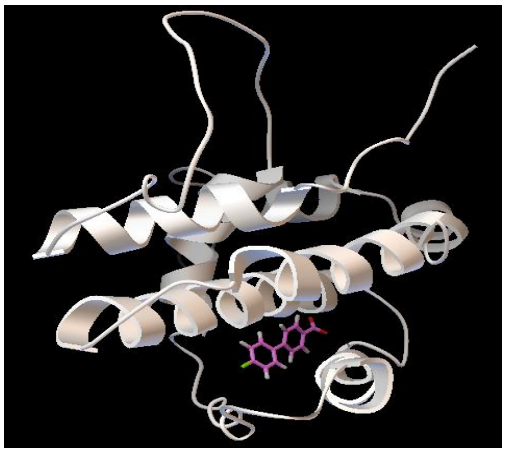


Fig 1.Bcl-xL protein with its ligand

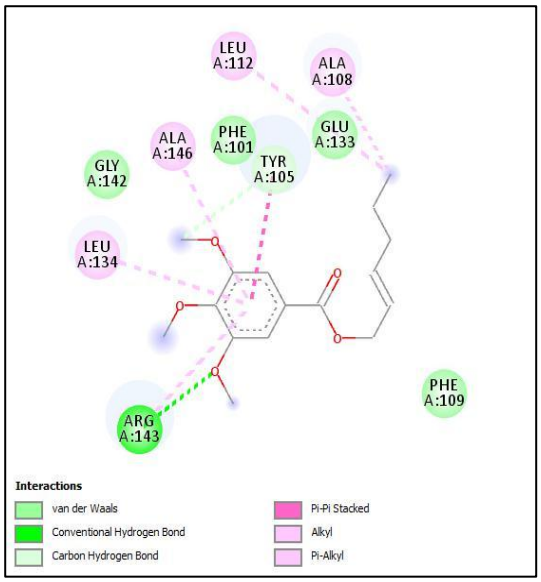
Before we used the docking methods, we need to validate the methods. We have evaluated the RMSD between the lowest energy docked Bcl-xL's ligand pose and the Bcl-xL's ligand native pose. From this validation, the RMSD is 1.296 Å.One generally considers an RMSD <2 Å as a successful prediction. With larger deviations, many of the observed interactions between the protein and the ligand will not have been predicted correctly[8].

Docking studies were performed to evaluate the effect of ligands on the macromolecules Bcl-xL. The top 5 of docking simulation results of gallic acid derivatives, gallic acid, and doxorubicin can be seen in table 3. Indicator from docking simulations can be seen by comparing the value of the Gibbs energy (ΔG) and inhibition constant. Gibbs energy (ΔG) showed the stability interaction between ligand and Bcl-xL residues, whereas inhibition concentration (Ki) was used to determine the binding energies of different docking conformation.

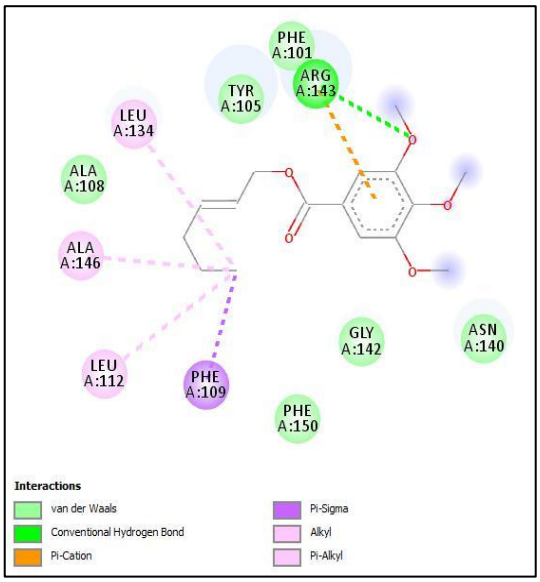
Table 3.Molecular docking interaction with Bcl-xL

No	Compound	ΔG (Kcal/Mol)	Ki (μM)
1	3,4,5-trimethoxy-*cis*-2-hexenylgallate **(19)**	-6.43	19.32
2	3,4,5-trimethoxy-*trans*-2-hexenylgallate **(18)**	-6.33	23.04
3	3,4,5-trimethoxy-hexylgallate **(17)**	-6.07	35.52
4	3,4-dimethoxy-*cis*-2-hexenylgallate **(16)**	-5.77	58.99
5	3,4-dimethoxy-*trans*-2-hexenylgallate **(15)**	-5.77	59.36
6	Gallic Acid	-3.51	2000.69
7	Gossypol	-5.77	61.37

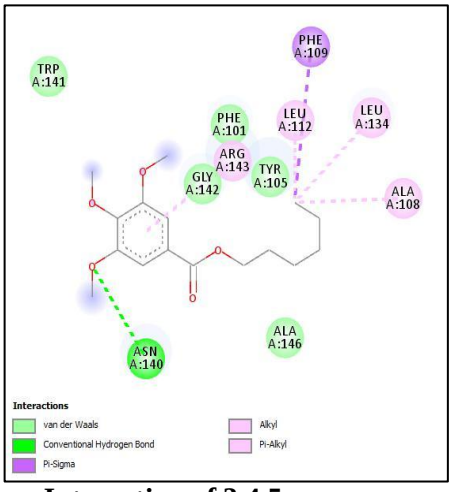
Based on the simulation results of docking above indicate that the best five gallicacid derivatives are 3,4,5-trimethoxy-*cis*-2-hexenylgallate;3,4,5-trimethoxy-*trans*-2-hexenylgallate;3,4,5 trimethoxyhexylgallate; 3,4-dimethoxy-*cis*-2-hexenylgallate; 3,4-dimethoxy-*trans*-2-hexenylgallate has good potential on the inhibition of Bcl-xL. Derivative compounds which have the greatest potential as a Bcl-xL inhibitor is 3,4,5-trimethoxy-*cis*-2-hexenylgallate, with ΔG value -6.34 kcal/mol and Ki value 22.47 μM. Compound **(19)** have better ΔG and Ki valuethanGossypol, with ΔG value -5.75 kcal/mol and Ki value 61.37 μM, which have anti-proliferative effects against breast cancer cell line.(-)-Gossypol also binds to the BH3-binding groove of Bcl-xL[12].This result shows that compound **(19)** is a potential inhibitor for Bcl-xL protein.



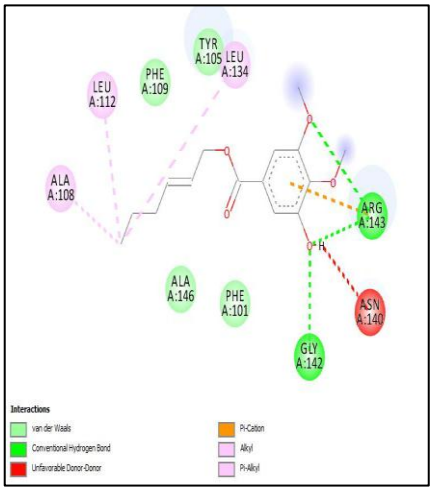
a. Interaction of 3,4,5-trimethoxy-*cis*-2-hexenylgallate withBcl-xL protein



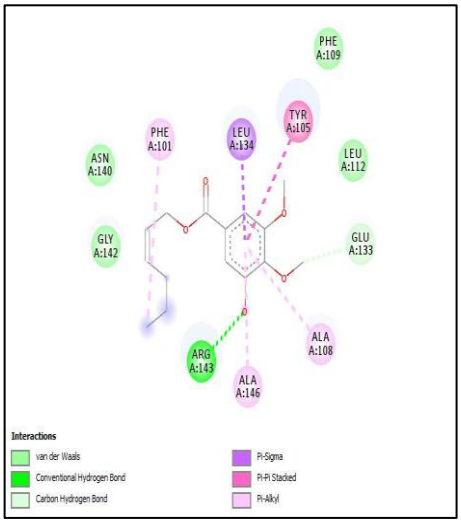
b. Interaction of 3,4,5-trimethoxy-*trans*-2-hexenylgallate with Bcl-xL protein



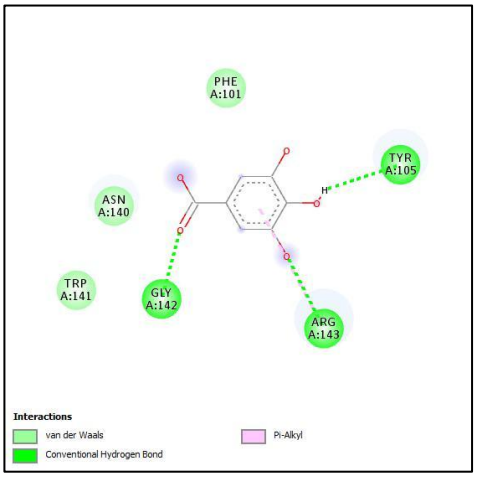
c. Interaction of 3,4,5-trimethoxyhexylgallate withBcl-xL protein



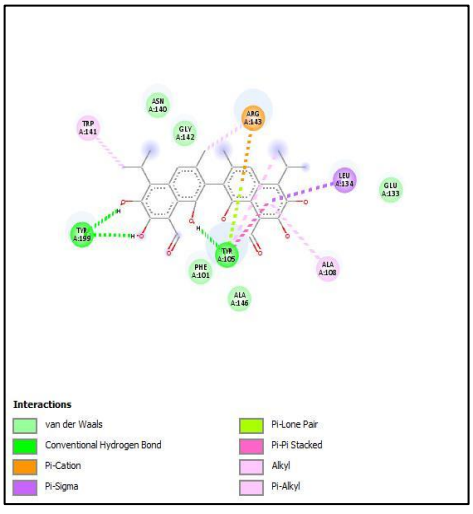
d. Interaction of 3,4-dimethoxy-*cis*-2-hexenylgallate with Bcl-xL protein



e. Interaction of 3,4-dimethoxy-*trans*-2-hexenylgallate with Bcl-xL protein



f. Interaction of gallic acid with Bcl-xL protein



g. Interaction of Gossypol with Bcl-xL protein

Fig 2a-c. Interaction between compound and macromolecule

Gallic acid and its derivatives were docked to Bcl-xL protein using Lamarckian genetic algorithm. PHE101, TYR105, ALA108, PHE109, LEU112, GLU133, LEU134, ASN140, GLY142, ARG143, ALA146 are the predominant residues lining the binding pocket. The compounds possess different poses in the active site according to its number of hydrogen bond interactions. Amino acid involved in the hydrogen bond interaction areTYR105, ASN140, GLY142, ARG143. ARG143 is the most common residue that involved in hydrogen bond (Fig. 2). Compound **(19)** has hydrogen bond interaction with ARG143, which ARG143 are the key residues form the "arginine fingers", the highly conserved residues. There is also a conserved "arginine finger" formed by ARG143 which plays an important role to induce the apoptosis. This compound displays strong pan-active inhibitory properties against Bcl-xL. The inhibitory constant Ki for protein-inhibitor binding was calculated which exhibits a strong correlation with binding energy. Lower Ki values directly relate to docking energy and inversely to the binding affinity that is inhibitor having the lower inhibitory constant shows the highest affinity to protein[5].From data above, compound **(19)**is satisfying Lipinski's rules, has lower (ΔG) and inhibitory constant, also has a hydrogen bond interaction with "arginine fingers", showed that compound **(19)** is a promising small molecule candidate against Bcl-xL protein that overexpress in breast cancer.

Conclusion

In summary, we contend that the compound **(19)** 3,4,5-trimethoxy-*cis*-2-hexenylgallate serve as potential inhibitor of anti-apoptotic Bcl-xL protein. Drugs based on small molecules docking studied here might be useful against breast cancer that over-express Bcl-xL proteins. The new findings are shaping our impressions on both possibilities and challenges for anticancer drug design. Need further in-vitro / in-vivo research with this new compounds that will give experimental IC_{50} values. Correlation between these parameters is helpful for further structure-based design of potent inhibitor.

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