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POTENTIAL INHIBITION OF AKT1 AND P53 PROTEIN IN COLON CANCER BY GALLIC ACID DERIVATIVES COMPOUND WITH MOLECULAR DOCKING APPROACH

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ABSTRACT
Colon cancer is a degenerative disease that attacks the large intestine through a process of initiation, promotion and progression. Related research reports that overexpression of the AKT1 protein was found to be 60-70% and p53 at 50%. This research analyses the affinity, stability and interaction of gallic acid derivative compounds and reference ligands with the target proteins AKT1 and p53 by molecular docking. The study stages carried out include preparation and optimization of target proteins and ligand compounds, file creation and simulation processes, and analysis and visualization of docking results. The docking simulation results show that four gallic acid derivative compounds provide potential inhibitory activity against the AKT1 and p53 proteins based on binding energy values. BG and 2HBG compounds have strong inhibitory power against target proteins, thus enabling the formation of strong interactions and complexity towards the active site of amino acids with a bond distance of <3.0 Å. Thus, gallic acid derivative compounds have potential as inhibitors and are expected to activate other proteins, causing cancer cell apoptosis.

Keywords: AKT1 and p53; Colon cancer; Gallic acid and its derivatives; Molecular docking

1. INTRODUCTION

Colon cancer is one of the most diagnosed and third-deadly diseases in the world and kills many every year (Nelson et al., 2020). methods generally use histopathological tissue analysis. APC (adenomatous polyposis cancer) gene mutations, delete in colon cancer (DCC), AKT, K-Ras, p53, BRAF serine/threonine proto-oncogenes, and damaged genes are markers of colon cancer development (Ahmed Monjur., 2020). Protein kinase AKT is a proto-oncogene from the serine/threonine kinase family that regulates several metabolic processes, cell mediators and controls cell survival (Revathidevi & Munirajan., 2019). In addition, it exerts influence on the promotion of cell cycle progression and inhibits apoptosis. AKT activity in highly active colon cancer ranges from 60 to 70% (Pandurangan., 2013) and is involved in the proliferation, metabolism, and survival of cancer cells (Zhu & Thompson., 2019). The compound MK-2206 showed inhibitory activity on the AKT protein in human colorectal cancer pre-clinical trials (Al-Saffar et al., 2018). AKT protein is a crucial and rational target in the development of anticancer drugs (Song et al., 2019). Research by Li et al, 2018 showed that the compound resveratrol provides excellent chemotherapeutic effects for AKT1 and AKT2 proteins in colon cancer (Li et al., 2019).

The p53 gene is a regulator of several biological functions of cells including proliferation, oncogene signaling, ribosomal stress, DNA damage, metabolism, DNA repair, differentiation,

and apoptosis, which also affects the process of carcinogenesis (Boutelle & Attardi., 2021). The expression of p53 in normal cells is at a low level because it is degraded by the ubiquitin ligase MDM2, Pirh2, and COP1 (Pant & Lozano., 2014). The p53 gene mutation in colon cancer is around 50%. The mutation occurs due to dysfunction of the p53 gene in tumors in the DNA-binding domain by hetero-oligomerization with evil-type p53 (Shah et al., 2020). This causes the cells to be oncogenic, leading to the invasion, proliferation, metastasis, and survival of cancer cells (Bergers & Fendt., 2021).

Gallic acid (GA) is a bioactive compound of the polyphenol group which is widely contained in various plants, vegetables, nuts, and fruit (Li et al., 2017; Bai et al., 2021). GA has several pharmacological activities including anticancer, antibacterial, anti-inflammatory, and antioxidant (Nouri et al., 2020; Kahkeshani et al., 2019). The activity of GA as an anticancer was reported by Wang et al, 2016 namely the inhibition of ROS-specific N-acetyl cysteine (NAC), further stimulating the apoptotic pathway via mitochondria in H446 SCLC cells (Wang et al., 2016). In addition, AG can inhibit the growth of several cell lines, namely HCT116 and HT-29 for colon cancer (Lin et al., 2021), as well as MCF-7 for breast cancer (Rezaei-Seresht et al., 2019).

The structure-activity relationship of gallic acid derivative compounds shows that hydroxyl group substituents, alkyl esters and the number of aromatic rings contribute to cytotoxic activity, solubility, lipophilicity and inhibition against cancer cells (AL Zahrani et al, 2020). This study became the basis for modifying the structure of the lead compound GA by adding hydroxy, alkyl groups and -OH substitution to methoxy. Thus, it is hoped that it can increase its lipophilicity and inhibitory activity against cancer target cells

The research approach is using the molecular docking method by analyzing the affinity, stability, and binding activity with molecular targets of protein. In addition, predictions of pharmacokinetic profiles for these compounds were also carried out.

2. METHODS

2.1. Tools and Material

The docking molecular study was carried out using an Intel Core i3-1115G4 computer with 8 GB of RAM. The software used is Chimera 1.10.2, MarvinSketch 15.5.11, PyMol 2.3.3, Autodock 4.2, passonline (way2drug) and Discovery Studio V21.1.0.20298. The material used is a gallic acid derivative which is designed and modified based on the structure and comparison compound gossypol (**Figure 1**) AKT1, and p53 target proteins downloaded from the Protein Data Bank (http://www.rscb.org/pdb/).

2.2. Method

The structure of the target protein in the form of AKT1 and p53 was traced through the website https://www.rcsb.id with PDB IDs 3MVH and 1XQH, respectively. The structure of the gallic acid-derived ligand was designed and fabricated using Marvinsketch software.

The first is the preparation of the target protein which includes tracing, downloading, optimizing, and separating from non-standard residues. The structure of the target protein was traced and downloaded from the PDB web with PDB-ID 3MVH (AKT1) and 1XQH (p53). The second is the preparation of the ligands which includes structure-based design and the manufacture of two-dimensional structures which are then converted into three dimensions, as well as the addition of hydrogen atoms and Gasteiger energy. The third is tethering the coordinates of Gridbox 40x40x40 with Grid center size x=17,485, y=-1,885, and z=27,403 for AKT1 and Gridbox 40x40x40 with Grid center x=8,217, y=-15.898 and z=11.33 for p53. Each ligand is in a flexible state that will interact with the target protein in a rigid state. Next, create a Grid Parameter File (GPF) and a Docking Parameter File (DPF) of the target protein complex with ligands. The last is the process of simulation, analysis, and visualization of docking results.

Pharmacokinetic studies were carried out on gallic acid derivatives and comparison ligands using the preADMET software via the website address https://preadmet.bmdrc.kr. The parameters measured were the permeability of Caco-2 cells to predict absorption in the intestine, the percentage of Human Intestinal Absorption (%HIA), and binding to plasma proteins to predict the distribution of these compounds.

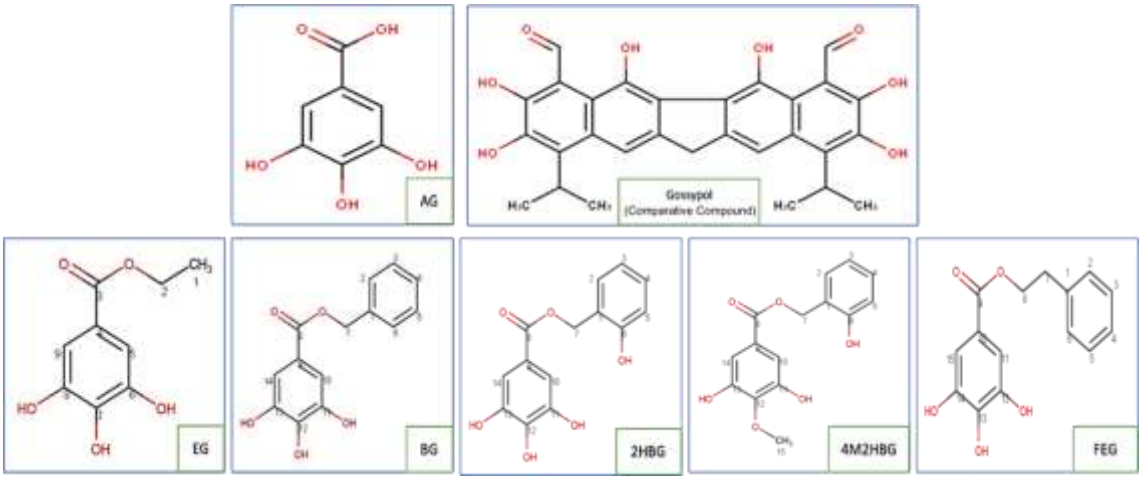

Figure 1. Structure Design of Gallic Acid Derivative Compounds (GA: Gallic acid; EG: Ethyl gallate; BG: Benzyl gallate; 2HBG: 2 hydroxy benzyl gallate; 4M2HBG: 4 methoxy 2 hydroxy benzyl gallate; FEG: phenylethyl gallate and Gossypol as ligand comparison)

3. RESULTS AND DISCUSSION

The study of molecular docking is performed on gallic acid derivative compounds with proteins that stimulate apoptosis, namely AKT1 and p53. The results of docking analysis are sorted by Affinity, stability, and bonding activity to the target protein and visualized to identify the bonding distance that occurs.

The relationship between the structural activity of gallic acid derivatives compound to both AKT1 and p53 target proteins gives varying binding energy values (**Figure 2** and **Figure 3**). Modification of the chemical structure by adding hydroxyl and methoxy groups greatly affects the inhibitory activity. In addition, gallic acid parent compounds consisting of benzene and hydroxyl groups play an important role in the activity of inhibition (Badhani et al., 2015). The number of hydroxyl groups affects the magnitude of inhibition effectiveness (Anantharaju et al., 2016). In addition, alkyl groups in the form of methoxy give the activity of the binding and inhibition are quite strong (Humaedi & Ernie., 2021).

Gallic acid derivatives compound used in the docking simulation for target proteins AKT1 and p53 met Lipinski's criteria, namely molecular weight <500 gram/mol, hydrogen bond proton acceptor group <10, proton donor group bonded hydrogen < 5, the logarithm of partition coefficient in water and 1-octanol < 5 (Chagas, Moss & Alisaraie, 2018). Compounds that meet these criteria are considered to have the potential to enter cell membranes and be absorbed by the body. Apart from that, based on way2drug.com analysis, it shows that this compound has anti-carcinogenesis activity with a Probability of Activines (pa) value > 0.3.

In silico studies regarding the activity of gallic acid derivatives against a certain target, proteins were reported by Kahkeshani et al (2019) and Variya et al (2012) that aryl-3,4,5-trimethyl gallate inhibits COX-1 as an anti-inflammatory and other gallic acid derivative compound as a strong inhibitor of PPAR-Y receptor as anti-diabetic. Gallic acid derivative compound, namely 2-hydroxy benzyl gallate, has inhibitory activity against BRAF protein in colon cancer (Humaedi et al., 2017). Research by Raghi et al, 2018 reported that the combined compound between gallic acid and 1,3,4-oxadiazole inhibited the activity of the ABL kinase receptor (Raghi et al., 2018).

Furthermore, 3,4,5 trimethoxy-phenylmethyl gallate compounds have the potential as inhibitors in prostate cancer by inhibiting the expression of androgen receptors (Humaedi & Ernie., 2021).

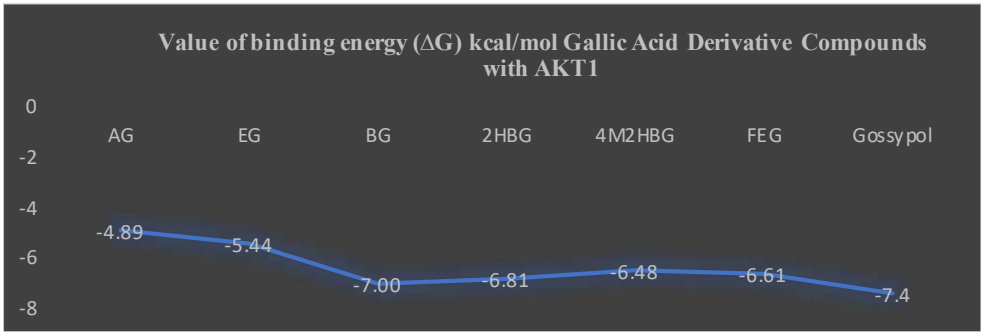

Figure 2. Value of binding energy (ΔG) kcal/mol of gallic acid derivatives compound with AKT1 (AG: Gallic acid; EG: Ethyl gallate; BG: Benzyl gallate; 2HBG: 2 hydroxy benzyl gallate; 4M2HBG: 4 methoxy 2 hydroxy benzyl gallate and FEG: phenylethyl gallate)

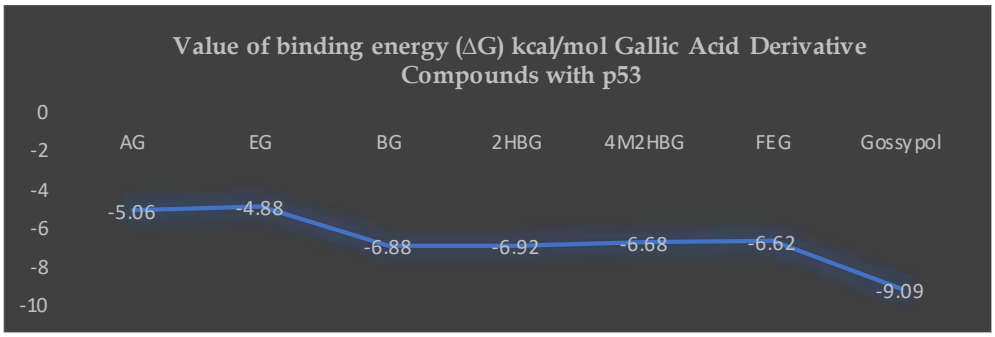

Figure 3. Value of binding energy (ΔG) kcal/mol of gallic acid derivatives compound with p53 (AG: Gallic acid; EG: Ethyl gallate; BG: Benzyl gallate; 2HBG: 2 hydroxy benzyl gallate; 4M2HBG: 4 methoxy 2 hydroxy benzyl gallate and FEG: phenylethyl gallate)

3.1. Docking Result Analysis with AKT1

The docking simulation data in **Figure 2** shows that four gallic acid derivative compounds provide the lowest binding energy values and have the potential as inhibitors for AKT1 protein, namely BG, 2HBG, 4M2HBG, and FEG. It has been validated before docking is carried out, with an Rmsd value < 2, namely akt1 is 1.77 and p53 is 1.154. The gallic acid derivative which has the best (ΔG) value is BG (-7.00 kcal/mol). Meanwhile, the comparison ligand, gossypol, gave a relatively higher inhibitory activity of -7.74 kcal/mol. This is possible because the molecular weight factor is large enough to affect its activity.

In addition to binding energy, other indicators that support the results of the docking simulation are the inhibition constant (μM), amino acids bound by hydrogen bonds, and bond distances. The smaller the value (Ki), the lower the binding energy so the greater the effect of inhibition. The interaction between the amino acids of the protein AKT1 with gallic acid derivative compounds formed a strong enough hydrogen bond with an average bond distance of 2.35 Å, which allows apoptosis through the activation of caspase 3 and 9. The stable hydrogen bond interaction analysis has criteria as hydrogen donor and acceptor with bond distance < 3.4 Å (Juhas & Zitko, 2020).

The Interaction Model between the four gallic acid derivatives compound and the comparison ligand with the AKT1 protein showed a favorable binding of hydrogen bonds and Van der Walls bonds. The compound predominantly binds to the amino acids Leu156, Ala230, and Glu228 to form hydrogen bonds (**Figure 4**). Chuang et al research, 2015 reported that A46 and a48 compounds based on in vitro activity test results provide excellent activity potential, followed by docking simulation of AKT proteins and hydrogen bonds with amino acids Ala230 and Asp292 as well as Thr211 and Ala230 (Chuang et al., 2015). Furthermore, imidazopyridine

derivative compounds exert inhibitory activity against the AKT1 protein and form interactions with the amino acids Thr211, Tyr272, and Asn53 for compound 44 as well as Thr211 and Tyr272 for compound 5 (Gu et al., 2019). Research by Rehan et al, 2014 describes the compound MK-2206 strongly binds to protein AKT1 on amino acids Asn53, Gln59, Leu78, Trp80, Val201, Tyr272 (Rehan et al., 2014).

3.2. Docking Result Analysis With p53

Figure 3 shows that four gallic acid derivative compounds provide the lowest binding energy values, namely 2HBG, BG, 4M2HBG, and FEG against the p53 target protein. The 2HBG compound gave a fairly good inhibitory effect of -6.92 kcal/mol. While the comparison ligand gossypol has a very strong inhibitory effect with a fairly high binding energy value of -9.90 kcal/mol. In addition to the molecular weight factor, the large number of hydroxyl and benzene groups in the Gossypol structure chain causes a large inhibitory effect.

Other criteria are the inhibition constant (μM) which is directly proportional to the binding energy value, amino acids are bound by hydrogen bonds and the bond distance shows a strong interaction (**Table 1** and **Table 2**) with an average bond distance of 2.32 Å. These interactions occur with the amino acids Glu, His, Glu, and Asn with different codes with strong bonds, both gallic acid derivatives and their comparison ligands (**Figure 5**). Research by Abbasi et al., 2015 reported that the interaction between the ligand PK7242 and p53 formed hydrogen bonds in the amino acids Leu145 and Thr230 with an average bond distance of 3.25 (Abbasi et al., 2015). Furthermore, Shah et al, 2020 explained that there was a strong interaction between the GK02723 ligand and p53 protein on His, Thr, Leu, Ser, Tyr, and Cys amino acids with varying codes (Shah et al., 2020).

Table 1. Molecular weights, inhibition constants, hydrogen-bonded amino acids. and average bond distances for AKT1 target proteins

Compounds	BM	Ki (μM)	Amino acids for AKT1	Average bond distance
AG	170.12	259.58	LYS_{179},GLU_{198},THR_{211}, GLU_{228},ASP_{292}	2.46 Å
EG	198.05	102.32	LYS_{179},GLU_{198}, THR_{211},ASP_{292}	2.59 Å
BG	260.06	7.87	LYS_{179},GLU_{198},ASP_{292}	2.37 Å
2HBG	276.23	10.17	LEU_{156},GLU_{228},ALA_{230},	2.24 Å
4M2HBG	290.07	17.67	Leu_{156}	2.19 Å
FEG	274.08	14.39	GLU_{228},ALA_{230}	2.37 Å
Gossypol	502.519	3.76	GLY_{159},ALA_{230}	2.08 Å

Table 2. Molecular weights, inhibition constants, hydrogen bonded amino acids and average bond distances for p53 target proteins

Compounds	BM	Ki (μM)	Amino acids for p53	Average bond distance
AG	170.12	196.53	SER_{225},HIS_{297},GLU_{356}	2.26 Å
EG	198.05	102.31	GLU_{356}	1.99 Å
BG	260.06	9.10	GLU_{228},HIS_{293}	2.05 Å
2HBG	276.23	8.87	GLU_{228},HIS_{293}, ASN_{296},HIS_{297},TYR_{335}	2.96 Å
4M2HBG	290.07	12.79	ASN_{296},TYR_{335},GLU_{356}	2.32 Å
FEG	274.08	14.09	GLU_{356}	1.94 Å
Gossypol	502.519	215.43	ASN_{282}, LYS_{294}, ASN_{296}, GLU_{356}	2.13 Å

3.3. Pharmacokinetic Studies

The results of pharmacokinetic tests explain that the four compounds with the lowest Gibbs energy meet the required parameters. These parameters, namely the ability of absorption with classification (1) poorly absorbed (0-20%), (2) moderately absorbed (20-70%), and (3) well absorbed (70-100%). Permeability ability with classification (1) low (<4 nM Sec-1), moderate (4-70 nM Sec-1), and high (>70 nM Sec-1). Protein-binding plasma with classification (1) is strongly

bound (>90%) and weakly bound (<90%) (Tabeshpour et al, 2018). The first parameter is Human Intestinal Absorption according to the criteria range of good absorption properties, namely 70-100%. Furthermore, the permeability of the compounds indicated was in the moderate category between 4-70 nM Sec-1. Finally, the measured plasma protein binding was very strong, namely three compounds with a value >90% and one compound approaching a value of 90% (**Table 3**).

Table 3. Predicted results of pharmacokinetic studies of gallic acid compounds. and their derivatives as well as comparative ligands

Compounds	Absorption		Distribution
	HIA (%)	Cell Permeability Caco-2 (nM Sec^{-1})	Plasma Protein Binding (%)
AG	53.70	13.85	65.38
EG	72.04	0.18	96.03
BG	86.46	17.24	95.83
2HBG	75.31	13.44	96.05
4M2HBG	86.12	14.00	87.82
FEG	87.15	19.00	95.19
Gossypol	84.40	20.86	100.00

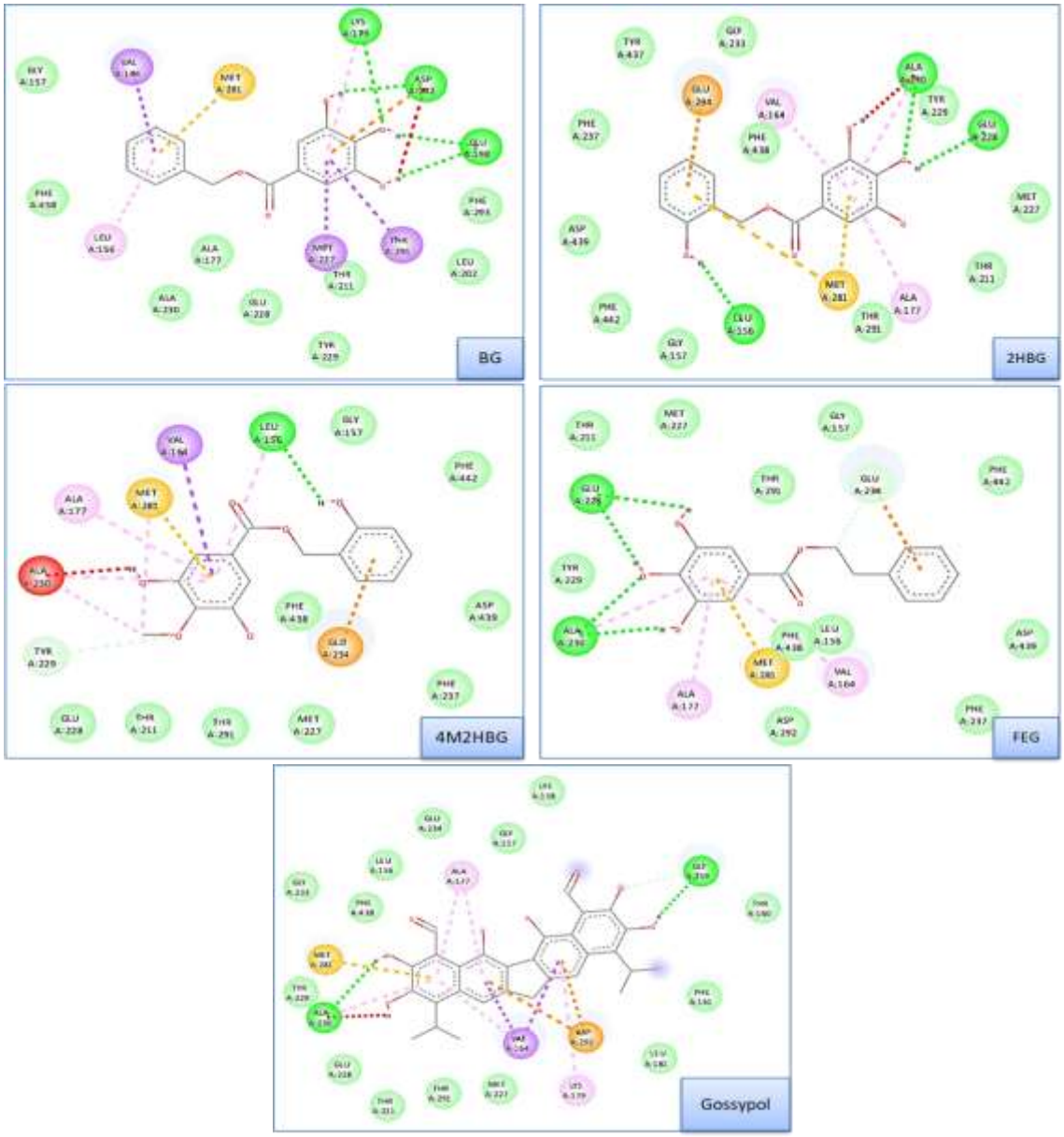

Figure 4. Interaction of AKT1 Protein with Gallic Acid Derivatives Compound (BG: Benzyl gallate, 2HBG: 2-hydroxy benzyl gallate, 4M2HBG: 4 methoxy 2 hydroxy benzyl gallate, FEG: phenylethyl gallate Comparative ligand: Gossypol)

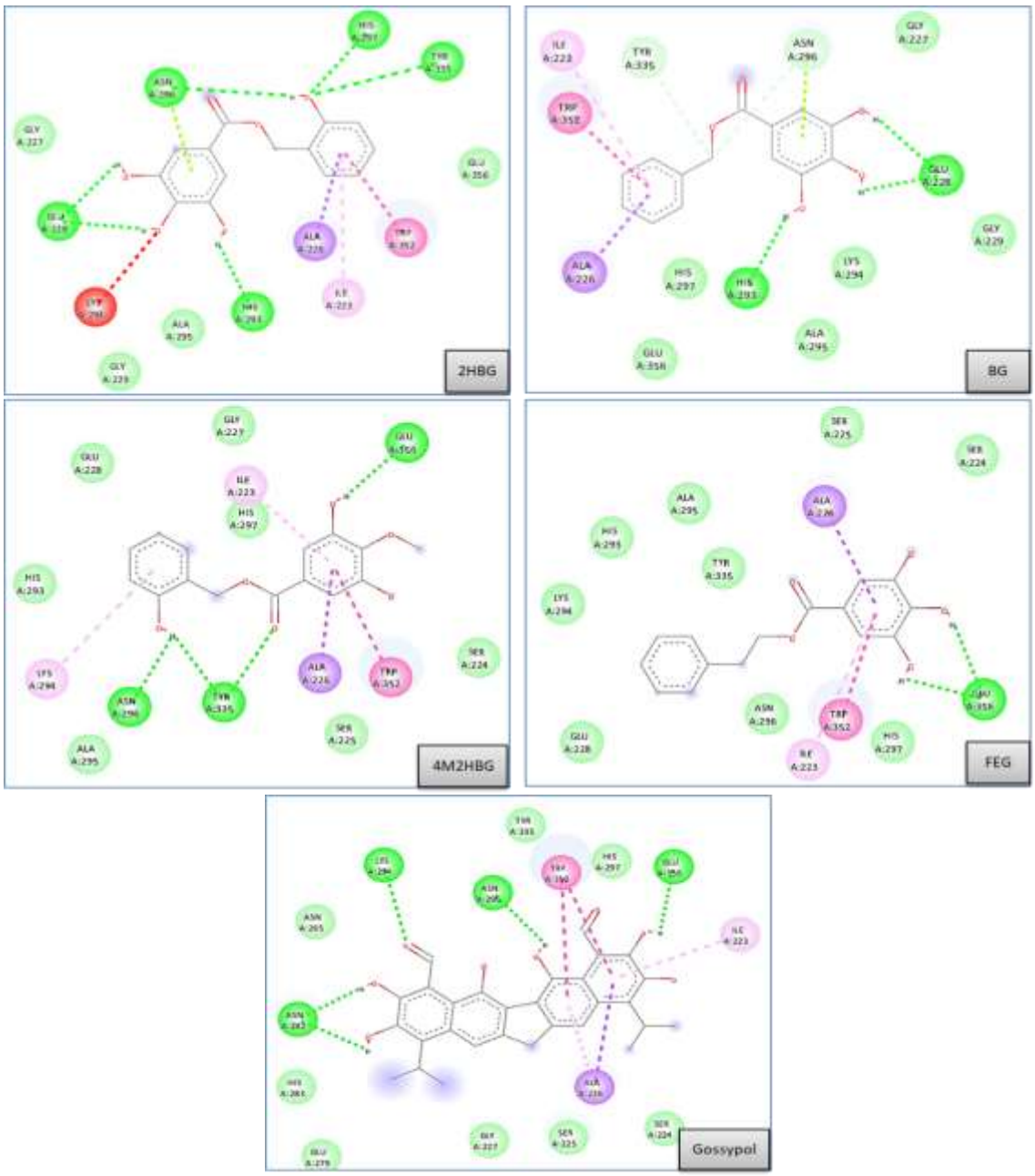

Figure 5. Interaction of p53 Protein with Gallic Acid Derivatives Compound (BG: Benzyl gallate, 2HBG: 2-hydroxy benzyl gallate, 4M2HBG: 4 methoxy 2 hydroxy benzyl gallate, FEG: phenylethyl gallate Comparative ligand: Gossypol)

4. CONCLUSION

This study shows that gallic acid derivative compounds have potential as colon cancer inhibitors by inhibiting the activity of AKT1 and p53 proteins with a docking simulation approach. Further research needs to be carried out using molecular dynamics to see the stability of the compound. Next, the synthesis and cytotoxic tests were carried out on this compound so that it has the potential to become a drug candidate for colon cancer.

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6. AUTHOR DECLARATION

Authors' Contributions and Responsibilities

The authors made substantial contributions to the conception and design of the study. The authors took responsibility for data analysis, interpretation, and discussion of results. The authors read and approved the final manuscript.

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Availability of Data and Materials

All data are available from the authors.

Competing Interests

The authors declare no competing interest.

Additional Information

No additional information from the authors.

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