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Research paper

Phytochemical Analysis and Study of Molecular Docking of *Leucaena leucocephala* Leave Extract as Anticancer

Analisis Fitokimia dan Studi *Molecular Docking* Ekstrak Daun *Leucaena leucocephala* Sebagai Antikanker

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ABSTRACT

Cancer is a disease caused by unnatural cell growth in the body. It has become a disease with a high mortality rate in the world. Several therapies have been developed for cancer treatment, one of which is the exploration of the bioactivity of compounds contained in potentially medicinal plants. One of the potential medicinal plants that grows in Central Kalimantan province is L. leucocephala. In this study, the potential of L. leucocephala leaf extract was explored through phytochemical tests and molecular docking approaches. Based on the phytochemical test, it was found that L. leucocephala leaf extract contains flavonoids, terpenoids, and steroids. Based on the test results, a literature study was conducted on the flavonoids and terpenoids content in L. leucocepala leaf extract and molecular docking studies were conducted. Seven dominant compounds of L. leucocephala consisting of flavonoids and terpenoids were tested for their potential as inhibitors of receptor tyrosine kinase. The molecular docking results showed that flavonoid ligands **(1-6)** had more negative binding affinity values compared to the drug gefitinib, a common drug for cancer therapy, while terpenoid ligand (**7**) had less favorable binding energy. Based on the Lipinski rule of five test, ligands **1**, **2**, **4**, and **5** fulfill all the criteria given as drug candidates, while ligands **3**, **6**, and **7** only fulfill **3** out of **5** criteria. Based on Lipinski rule of five and molecular docking, ligand 1,2,4, and 5 has potential to be anticancer.
Keywords:*: anticancer, molecular docking, Leucaena leucocephala, phytochemical.*

PENDAHULUAN

Cancer is a health disorder that can be caused by abnormal and potentially invasive cell growth . One technique for cancer therapy is chemotherapy (Devi et al., 2015). However, chemotherapy has side effects and often affects the tissues or organs around cancer cells (Love et al., 2013). Based on this, scientists are encouraged to find other methods to carry out cancer healing therapy. One of the therapies explored is the use of natural compounds found in plants.

Indonesia is a country with rich biodiversity. Likewise in the province of Central Kalimantan which has an abundant

biodiversity. This increases attention in the exploration of plant utilization as a medicinal material. One of them is *Leucaena leucocephala* which grows in Central Kalimantan. *L. leucocephala* is a type of legume plant that is spread in tropical regions such as Indonesia (Abu Zarin et al., 2016; Jayanthy et al., 2014). In previous studies, the active compound content of seeds from *L. leucocephala* has been successfully isolated and contained several compounds such as β-sitosterol, β-sitostenone, stigamstenone, isovalinilic acid, and methyl paraben (Chen & Wang, 2011). In addition, several researchers have also explored extracts from *L. leucocephala* leaves that contain several flavanoids that have antioxidant activity (Hassan et al., 2014). The use of *L. leucocephala* from seed extract has been reported to be antidiabetic (Chowtivannakul et al., 2016). However, there are still few studies that explore the content of *L. leucocephala* leaves extracts as anticancer.

Flavanoids are one of the natural ingredients that have many hydroxyl groups and have potential as antioxidants (Lin et al., 2014; Yahfoufi et al., 2018). The role of flavonoids as antioxidants provides an important role in controlling the level of oxidative stress caused by the abundance of reactive oxygen species (ROS) in several types of cancer (Devi et al., 2015; Marzocchella et al., 2011; Soleimani & Sajedi, 2020). Several studies have reported that flavonoids have properties as inhibitors of receptor tyrosine kinases (RTK) in the tyrosine kinase signaling pathway (Imran et al., 2019). Some proteins that act as RTKs are Epidermal Growth Factor receptor (EGFR) (Golonko et al., 2019; Mele & Johnson, 2019).

Understanding the interactions of drug molecules on the way to reach the target cancer receptor plays an important role in developing anticancer compounds. Several studies were conducted to study this, one of which used molecular docking approach and the use of Lipinski rule of five for drug molecules (Eberhardt et al., 2021; Lipinski, 2004; Trott & Olson, 2009). Therefore, in this study, phytochemical test of *L. leucocephala* leaves extract content and prediction of the potential content of active compounds as anticancer using molecular docking approach were conducted.

METHODS

Material

In this study, the material was divided into two categories, for phytochemical study and in silico study. The material used for phytochemical study were *L.leucocephala* leaves get from Central Kalimantan Provinces, solevet from Merck (methanol, ethyl ethanoat, *n*-hexane), Meyer reagen (merck), hydrochloric acid (HCl), acetic acid anhydride ($(CH_3CO)_2O$), iron (III) chloride ($FeCl_3$), magnesium powder, and sulfuric acid (H_2SO_4).

The material used for in silico study were ligand downloaded from PubChem database (https://pubchem.ncbi.nlm.nih.gov/)/ Ligand used in this study based on the experiment of (Xu et al., 2018). Protein target downloaded from RCSB Protein Data Bank (https://www.rcsb.org/).

Hardware and Software

The in-silico study was conducted on PC with processor Intel Core i5 7200U, RAM 8 GB, GPU 2 GB. Autodcok vina, PyThon, and Biovia Discovery Studio were used to conduct molecular docking and interaction analysis. PASS Online Test is used to predict the activity of the ligand in inhibiting, preventing, and killing the growth and spread of the cancer. The Lipinksi rule of five was determined using Lipinski rule's online prediction in admetSAR (http://lmmd.ecust.edu.en/admetsar2).

Procedures

Phytochemical test for Leucaena leucocephala leaves extract

Leaf samples were dried and mashed. Then, for extraction, the leaf sample was weighed 2 kg and put in a dark-colored bottle and added 3 L of ethanol solvent. The sample was left for 2x24 hours. After that, the samples were filtered, and the filtration results were concentrated. Sample extract 1 mL was taken, and n-hexane 1 mL was added, then shaken and allowed to stand until two layers were formed. The hexane layer was used for terpenoid and steroid tests. Terpenoid test was done by adding 3 drops of sulfuric acid to the n-hexane extract. On the other hand, for the steroid test, one drop of acetic anhydride and concentrated sulfuric acid were added. Phytochemical testing of flavonoids, phenolics, and alkaloids, ethanol extract was used. 3 drops each of ethanol extract was added with Mg powder and HCl for the flavonoids test, iron

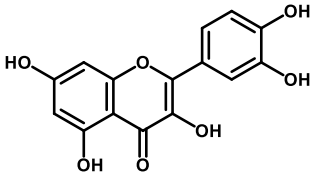
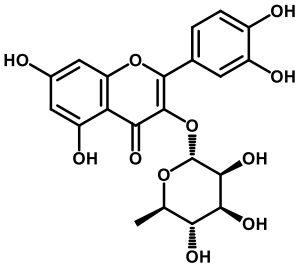
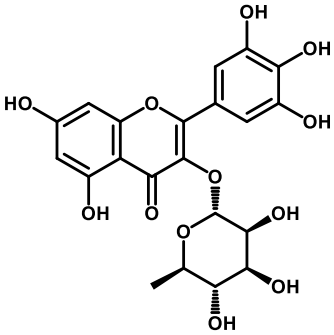
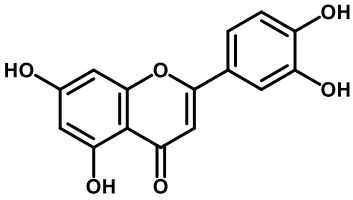
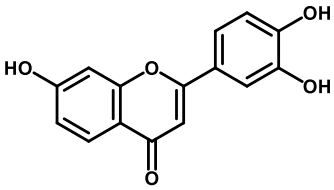
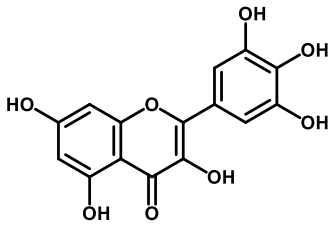
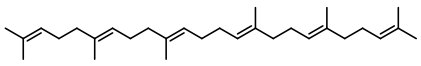
(III) chloride for the phenolics, and Meyer's reagent for the alkaloids.

Collection of active compounds of Leucaena leucocephala

The active compound of *Leucaena leucocephala* is collected based on the Xu, et. al. and Zayed experiment. The active compound list was available in Table 1**.** The active compound was downloaded from PubChem (https://pubchem.ncbi.nlm.nih.gov/). The prediction of compound activity were predicted using PASS online via website (http://www.way2drug.com/passonline/). The results of the PASS test for each active compounds are Pa (potential activity) and Pi (potential inhibitor) values based on the activity of similar compound structure with the active compounds.

Lipinski rules of five is determined the ability of active compounds as a drug to penetrate cell membranes and reach the target. The Lipinski rules of five was test using online web (http://www.scfbio-iitd.res.in/software/drugdesign/lipinski.jsp) by insert the pdb file of active compounds.

Table 1. Active compound in *Leucaena leucocephala* leaves

Compound	Structure
1	 PDB ID: 5280343
2	 CID: 481107531
3	 CID: 198991140
4	 CID: 5280445
5	 CID: 5322065
6	 CID: 5281672
7	 CID: 638072

Molecular docking and visualization

The molecular docking was conducted using Autodock Vina and visualized by Biovia Discovery Studio. Common anticancer drug such as sunitib were used as a positive control. The protein target was downloaded from RCSB PDB. Protein target used in this study is EGFR (PDB ID: 1M17). Furthermore, the water molecules and ligand on receptor were removed because it not needed in the experiment. After that, the protein structure was saved in .pdb format, which can be used in the docking simulation.

RESULTS AND DISCUSSION

L. leucocephala leaves extract has been successfully obtained and tested for phytochemicals to determine the type of active compounds. The result is available in Table 2. Based on the results of the phytochemical test, it is known that *L.leucocephala* leaves extract contains flavonoids, terpenoids, and steroids. The content results are in accordance with the research reported by Xu, et. al. and B that the content of *L. leucocephala* extract is mostly flavonoids and terpenoids (Xu et al., 2018 and Zayed & Samling, 2016).

Table 2. Phytochemical test leaves extract of *L. leucocephala*

No	Active compounds	Test Results
1	Terpenoid	+
2	Steroid	+
3	Flavonoid	+
4	Phenolic	-
5	Alkaloid	-

There are 7 active compounds that are dominant in Leucaena leucocephala leaf extract. These seven compounds are 6 flavonoid compounds (**1-6**) and one terpenoid compound (**7**). The seven compounds were predicted for their activity as anticarcinogenic through PASS online to obtain Pa and Pi data as in Table 3. The Pa and Pi values generated from PASS online are based on the activity of other compounds that have similar structures to the drug compounds in the data bank. Pa indicates potential activity, while Pi indicates probability to be inactive. Activity is said to be strong if the value of Pa>0.7, moderate if 0.5<Pa<0.7. In the online PASS prediction results, flavonoids **1, 2**, **3**, and **6** are classified as having strong activity, while flavonoids **4** and **5** are classified as moderate. Terpenoid compound **7**, has low anticarcinogenic activity (Pa < 0.5). Nevertheless, compounds with low predicted activity values (Pa) can be caused because these compounds have not been widely explored for their potential.

The results of the molecular docking simulation are the binding affinity value and the interaction pose between the ligand and the receptor at the active site of the target protein. The more negative the binding affinity value, it can be said that the interaction between the ligand and the receptor protein is more stable. The resulting ligand-receptor interaction can be in the form of hydorgen bonding interaction, hydropobic interaction, or electrostatic interaction. The results of binding affinity and amino acid residues resulting from ligand-receptor interactions are presented in Table 3 and Table 4. The seven test ligands have different binding affinity values depending on the interaction that occurs between the ligand and the receptor. The interaction that most affects the binding affinity value is hydrogen bonding, but other interactions also affect the binding affinity value.

Table 3. Binding energy and activity prediction as anticancer

Comp	Energy Binding (Kcal mol^{-1})	PASS Prediction	
		Pa	Pi
1	-8.9	0.757	0.007
2	-9.2	0.943	0.002
3	-9.2	0.955	0.001
4	-8.6	0.690	0.009
5	-8.4	0.603	0.012
6	-8.9	0.784	0.006
7	-7.0	0.445	0.025
gefitinib	-8.6	-	-

Molecular docking simulation begins with validating the method using redocking. The native ligand in the protein structure was taken and redocked. The method is said to be successful if the native ligand can be bound back to the active site and the RMSD value < 2.0 A. In this study, the RMSD value of redocking is 0.239 A, so it can be said that the method used has been valid.

In Table 3, the binding affinity data of the seven test ligands is provided. Compounds 1-6 have more negative binding energy values compared to gefitinib as the control ligand. Based on these results, it can be said that the test ligands have better interaction stability compared to gefitinib. In Table 4, it can be involved that the test ligand compounds 1-6 were able to form more hydrogen bonds compared to gefitinib, so it can be said that hydrogen bonds have a significant effect on the binding affinity value.

In addition, the potential of the test ligands as drug candidates was also assessed based on the interaction of the ligand with amino acid residues on the active side of the protein. The similarity of the amino acid residues of the control ligand and the test ligand indicates the ability of the test ligand to inhibit the activity of the target protein and has potential as a drug. Furthermore, the ability of the test ligand to form interactions with important amino acid residues on the active side also increases the potential of the test ligand as a drug candidate. The interactions of test ligand and gefitinib with receptor is available in figure 1.

Gefitinib as a control ligand is able to form hydrogen bonds with several amino acid residues such as Met769, Asp831, and Lys721. Test ligands **1-6** were mostly able to form interactions with all three amino acid residues. However, in compound **7**, not a single hydrogen bond formed with the target protein was detected. This is because in the structure of compound **7**, there are no atoms with high electronegativity.

Table 4. Amino acid and ligand interaction with EGFR

Comp	Hydrogen bonding	Hydrophobic bond
1	Met769, Gln767, Asp831, Glu738, Lys721	Gly772, Leu768, Thr830, Thr766, Phe832, Leu764, Met742, Ala719, Lys721, Leu820, Val702, Gly695, Leu694
2	Leu694, Gly772, Thr766, Arg817, Asp831	Phe699, Asn818, Lys721, Cys773, Met742, Thr830, Ala719, Leu820, Val702, Leu768, Pro770, Leu694
3	Lys721, Asn818, Asp831	Leu694, Met769, Leu768, Val702, Thr766, Ala719, Leu764, Met742, Glu738, Phe832, Gly695, Thr830, Phe699, Arg817, Leu820, Cys773, Gly772, Pro770
4	Met769, Thr830, Asp831	Leu764, Glu738, Met742, Thr766, Lys721, Leu820, Ala719, Leu768, Gly772, Leu694, Val702
5	Glu738, Thr830, Asp831, Met769	Met742, Leu764, Lys721, Leu820, Ala719, Thr766, Leu768Gly772 Leu694, Val702
6	Glu738, Thr830, Asp831, Gln767, Met769	Met742, Leu&64, Lys721, Leu820, Val702, Ala719, Leu694, Gly772, Pro770, Leu768, Thr766
7	-	Gly772, Leu820, Val702, Lys721, Ala719, Thr766, Met742, Leu764, Thr830, Asp831, Arg817, Asn818, Phe699, Cys773, Leu694
8 (gefitinib)	Met769, Asp831, Lys721	Val702, Leu694, Gly772, Leu768, Gln767, Leu820, Ala719, Thr766, Leu753, Leu764, Glu738, Thr830, Phe832, Met742, Phe699, Pro770
Native ligand	Met769	Pro770, Phe771, Gly772, Leu768, Leu820, Ala719, Gln767, Leu764, Thr766, Met742, Lys721, Thr830, Glu738, Asp831, Val702, Leu694, Gly695

Lipinski rule of five analysis is usually used to predict the bioavailability of a compound (ligand) to become a drug compound. The Lipinski test aims to explain the ability of the compound to penetrate into the cell membrane and reach the target receptor. Based on these

rules, a compound has the potential to become a drug if it meets at least two of the five rules including (1) molar mass cannot be more than 500 (Mw < 500); (2) cannot have hydrogen bonding acceptor >10; (3) cannot have hydrogen bond donor value >5; (4) log-P value less than 5, and (5) molar refractivity value between 40-130. Prediction of Lipinski's for ligand test is available in Table 5. Lipinski's prediction of the seven ligands showed that all seven ligands met at least three criteria (compound **7** did not meet the Pa and Molar refractivity criteria). As a positive control, gefitinib, a cancer drug, fulfills all five Lipinski's criteria.

Table 5. Lipinski rules analysis of ligand with positive control

Comp	MW	HBD	HBA	LogP	MRef
1	302	5	7	2.01	74.05
2	312	5	6	-0.05	77.15
3	444	0	12	-0.83	73.32
4	286	4	6	2.12	72.48
5	270	3	5	2.42	70.81
6	318	6	8	1.72	75.72
7	410	0	0	10.61	140.06
gefitinib	447	2	6	1,56	115.18

Mw = molecular weight;
HBD = Hydrogen Bonding Donor;
HBA = Hydrogen Bonding Acceptors;
LogP = octanol-water partition;
Mref = Molar refractivity

Predictive studies on pharmacology through Lipinski rules are very important because a drug compound will work if it reaches the target receptor. When a compound is orally administered, it will be absorbed in the intestinal wall and flow with the bloodstream, and distributed in the body to reach the target receptor. Compounds with a molecular weight of more than 500, are predicted to have difficulty to penetrate the cell membrane, while compounds with a lower molecular weight will enter more easily through the diffusion process. All seven test ligands met these criteria (Mw < 500).

According to penetration of compound to membran cell, the HBA and HBD values of drug candidtate must be less than 10 and 5, because if the values are too large, it will slow down the rate of the compound to penetrate cell membrane. In this criterion, compounds **3** (HBA>10) and **6** (HBD>5) did not meet the criteria of Lipinski. When viewed from the structure of compounds **3** and **6**, both compounds have more hydroxyl groups than the other test compounds, so it is possible to have HBA and HBD values that do not meet Lipinski's criteria.

logP values indicate the hydrophilic and hydrophobic nature of the compound. If the logP value is negative, it means that the compound is hydrophilic, otherwise if the value is more positive, it means that the compound is increasingly hydrophobic. Drug compounds that have a high logP value (>5), will easily enter the lipid bilayer on the cell membrane. This causes the compound to be over-distributed and selectivity to the target receptor is reduced. In this study, compound **7** has a logP value that does not meet the criteria because it is 10.61. Based on the structure of compound **7**, this is possible because compound **7** is a hydrocarbon that tends to be nonpolar, so it will be hydrophobic.

Based on the analysis of Lipinski rules, binding affinity, and similarity of interactions with the control ligand, compounds 1,2,4, and 5 have potential as drug candidates because they meet the five criteria of the Lipinski rule, have a more negative binding affinity than the control ligand which is as high as -8.9, -9.2, -8.6, and 8.4 kcal mol^{-1}, and most of amino acid residue has similarities with interactions on the control ligand.

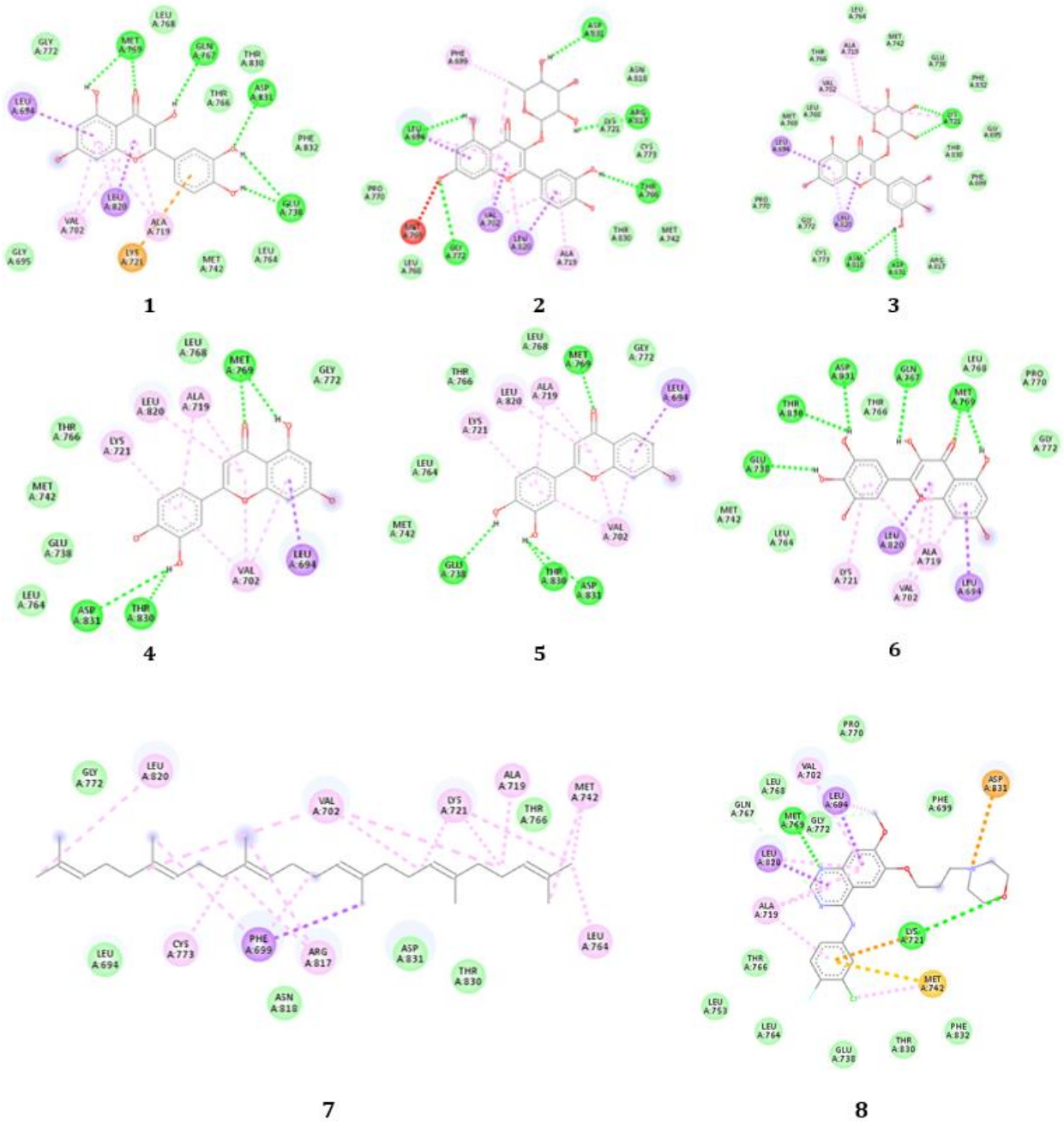


Figure 1. Interactions of ligand test and ligand control with protein target

CONCLUSSION

Phytochemical tests, prediction of drug candidates through Lipinski rules, and molecular docking studies have been carried out in this study. Through phytochemical tests, it is known that Chinese petai, which grows in the Central Kalimantan province, contains flavonoids, terpenoids, and steroids. Based on 7 test ligands derived from active compounds in Chinese petai leaf extracts, they meet at least 3 criteria as drug candidates based on Lipinski rules of five. Compounds 1, 2, 4, and 5 fulfill all five Lipinski rules criteria. Molecular results show that compounds 1-6 have more negative binding affinity compared to the control ligand, gefitinib. So it can be said that compounds 1-6 have a better interaction than gefitinib. Furthermore, compounds 1-6 have similar interactions with the amino acid residues of the target compound. Based on the molecular docking results and Lipinski rule study, compounds 1,2,4, and 5 have strong potential as anticancer because they have more negative binding affinity and fulfill all Lipisnki rule criteria.

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