# Molecular Docking of Mangrove Plantas Therapeutic Agentto Treat Non-Small Cell Lung Carcinoma

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## ABSTRACT

**Background:** Cancer is one of the biggest health problems worldwide, with lung cancer as the first rank in the number of new cases and deaths. Non-small cell lung carcinoma (NSCLC) is a type of lung cancer that accounts for about 85% of all lung cancer cases. Previous research identified the role of epidermal growth factor receptor (EGFR) as the most suitable target to treat NSCLC. This study aims to identify the potential compounds derived from mangrove plants as agents to treat NSCLC using a molecular docking study.
**Methodology:** Six natural compounds, which include taraxasterol, stigmasterol, tretinoin, heritonin, ascochitine, and tricin, along with gefitinib as a drug comparative were used. Docking was carried out on EGFR as a receptor target by Autodock Tools. The visualizations of molecular interactions were carried out by BIOVIA Discovery Studio 2020.
**Results:** The results showed that all six compounds were compiled from several criteria as drugs based on Lipinski analysis and had an affinity to EGFR receptors. The docking results were found in the order of stigmasterol (-11.84 kcal/mol), taraxasterol (10.80 kcal/mol), tretinoin (-10.60 kcal/mol), tricin (-9.24 kcal/mol), ascochitine (-7.85 kcal/mol), heritonin (-7.81 kcal/mol), and gefitinib (-8.62 kcal/mol). Among these natural compounds, stigmasterol exhibited the highest binding affinity. ADME profile showed that these natural compounds are safe and drug-like compounds.
**Conclusion:**Stigmasterol exhibited the highest binding energy of -11.84 kcal/mol. All three compounds bind in the binding pocket of EGFR. All compounds have drug-likeness properties based on Lipinski rules



*Keywords: Epidermal growth factor; molecular docking; non-small cell lung carcinoma; taraxasterol; stigmasterol.*


## 1. INTRODUCTION

Cancer is one of the biggest health problems that lead to the leading causes of death worldwide[1]. This disease also shows an increasing trend in recent years and is predicted to increase every year[2].Lung cancer is the leading cause of cancer death among both men and women in the United States so far. In 2022, American Cancer Society estimated 236,740 new casesof lung cancer will be diagnosed and 130,180 will die, approximately 350 deaths per day caused by lung cancer, the leading cause of death[3].

Lung cancer has been linked to several factors including smoking, genetic predisposition, and environmental factors[4]. Lung cancer can be classified into two types which are small

cell lung cancer (SCLC) and non-small cell lung carcinoma (NSCLC), with NSCLC responsible for approximately 85% of lung cancer cases[5]. Lung cancer mayexist because of genetic and epigenetic changes of thecellular genome, which is critical to the disease progression. The comprehensive molecular dissection of NSCLC found the mutation in epidermal growth factor receptor (EGFR) genes for about 10-40% cases with 14-19% of western patients and 40-48% of Asian patients. EGFR is a kind of tyrosine kinase receptor located at the cell surface. EGFR can generate differentiation and proliferation of cells upon activation through the binding of one of its ligands. Based on the fact that EGFR mutation leads to NSCLC, research has shown that targeting EGFR is currently considered the most suitable way to treat it[6,7].

The current treatments which are usually used to treat NSCLC are surgery,chemotherapy, and targeted therapy[8]. NSCLC patients whose tumors activate kinase domain mutations in EGFR often respond to EGFR tyrosine kinase inhibitors (TKI) such as erlotinib, gefitinib, and afatinib[9]. However, some studies have found TKI drug resistance in some NSCLC patients with EGFR mutation[10]. NSCLC treatment with surgery is invasive and limited to stage I-II and IIIA[11] Moreover, chemotherapy treatment which is often used in several types of cancer, had serious side effects[12] Therefore, the development of novel and treatment for treating NSCLC patients is needed. In the last few decades, research on herbs as an alternative treatment with minimal side effects has been developed. Natural compounds are widely used in various therapeutic interventions due to their benefits as anticancer and minimum side effects[13,14].

Mangrove plant has abundant bioactive compounds and can serve as a reservoir for novel bioactive compounds suchas amides, alkaloids, tannins, flavonoids, saponin, glycosides,terpenoid, phenolic, and phytosterol [16]. The pharmacological activities of terpenoid, phenolic, and phytosterol indicate that they have a potential as preventative supplements and pharmaceutical agents as anticancer, antifungal, antibacterial, antiviral, antioxidant, anti-inflammatory, and other activities. Some specific compounds pose prominent effects as anti-cancer. Terpenoid exhibits cytotoxic against manycancercell lines[17,18].Taraxasterol is one of terpenoid known as anticancer for a few types of cancer. Chen et al. revealed that taraxasterolcut the growth of gastric cancer by inhibiting of EGFR signaling[19].The main phytosterol in mangrove, stigmasterol was also found to inhibit proliferation and promoted the apoptosis of lung cancer cells [20]. As a whole, Song et al.reported that mangrove compounds can be a multi-target inhibitors such as inhibit activities of HER2, HER3, HER4, RET, and EGFR in treating NSCLC[21].The specific compounds that play a role in inhibiting EGFR remain unclear. Thus, the present study aims to identify the potential compounds derived from mangrove plants as therapeutic agents to treat NSCLC.

## 2. MATERIALS AND METHODS

This study was conducted utilizing molecular docking computational method. The materialsused in this study were protein target, namely EGFR (PDB ID: 3G5Z) downloaded from http://www.rcsb.org and mangrove compounds weredownloaded from https://pubchem.ncbi.nlm.nih.gov. The drug used as comparative was Gefitinib (Compound CID: 123631) which downloaded from https://pubchem.ncbi.nlm.nih.gov.

### 2.1 Selection of Mangrove Compounds

The mangrove compounds were retrieved from previous studies. Six mangrove compounds were used in this study including taraxasterol (Compound CID:115250), stigmasterol

(Compound CID: 5280794), tretinoin (Compound CID: 444795), heritonin (Compound CID: 130118), ascochitine (Compound CID: 73486), and tricin (Compound CID: 5281702).

## 2.2 Geometry Optimization

After the compounds from Pubchem were downloaded, the compounds were saved in pdb format using Discovery studio. Then, geometry optimization was carried out in Argus Lab 4.0.1. software using PM3 semi-empirical parameterization based on Hartree-Fock calculation method. Argus lab software computed the energy convergence (stopping pointof the compound's molecule[22].Furthermore, the compound'sformat was converted to pdb with OpenBabel software to make it readable with Autodock Tools program[23].

## 2.3 Preparation of Target Protein and Compounds

The target protein was used in this study was EGFR (PDB ID: 5UG8). The preparation of the target protein was performed by removing water molecules (H2O) contained in the target protein,adding polar hydrogen atoms, cleaning the target protein structure from natural ligands then saved its file in the pdbqt format[24]. The preparation of the compoundswerecarried out bychangingsdf format to pdbqt format using Discovery Studio and AutoDock software.

## 2.4 Validation

Validation of the molecular docking method was done by redocking the native ligand (N-[(3R,4R)-4-fluoro-1-{6-[(1-methyl-1H-pyrazol-4-yl)amino]-9-(propan-2-yl)-9H-purin-2yl}pyrrolidin-3yl]propenamide) to the selected macromolecule (EGFR) using Autodock Tools software. The binding site and the parameters used in this study are considered valid the RMSD value is ≤ 2Å[25].

## 2.5 Docking Protocol

The molecular docking was carried out to predict the binding energies of the compounds toward target protein using the Autodock Tools, Autogrid4, and Autodock4 software[26,27].The docking simulation was done by arranging the docking parameters, which are the grid box size (x = 40, y = 40, z = 40), the grid box coordinate (x = -13.156, y = 14.7, z = -25.718), 0.375Å spacing, 100 runs, medium number of evals, and Lamarckian Genetic Algorithm 4.2 The docking output is indlg format. The lowest binding affinity was selected from a set of 100 conformation poses.The interactions which exhibit the strong binding energy were analyzed using Discovery Studio software.

## 2.7 Drug-likeness and Toxicity Analysis

The Lipinski rule of five was used in this study assess the drug-like properties of compounds[28].The molecular weight, number of hydrogen donor and acceptor, solubility, permeability,level of GI absorption, and number of Lipinski violations were screened byemploying the Swiss ADME web tools http://www.swissadme.ch/index.php[29]AdmetSAR 2.0 online tool(http://lmmd.ecust.edu.cn/admetsar2) was used to predict the toxicological profile of selected compounds[30].The finalized ligands' SMILES were submitted in the admetSAR website to check for toxicity[31].

## 3. RESULTS AND DISCUSSION

### 3.1 Molecular Docking Study

All compounds were docked to analyze their binding energy using Autodock Vina. Analysis of the molecular docking results were carried out by assessing the binding energy (ΔG).The validation was performed by redocking the native ligand to EGFR, using the determined parameters, showed the RMSD value of 1.91 Å.Since the value is less than 2 Å, the docking method can be used to dock the test compounds. The difference between the native ligand before and after the redocking procedure (Fig. 1). The docking results are represented in Table 1. Stigmasterol, taraxasterol, and tretinoin were found to have the highest binding energy of -11.84, -10.80, and -10.60 kcal/mol,respectively. The comparative drug, gefitinib was found to have binding energy of -8.62 kcal/mol.Hence, the ability of the compounds to bind EGFR was more exceptional. They showed that more robust and stable interactions that occur between the compounds and EGFR [32]In addition, the binding energy value is directly linear with the constant inhibition value (Ki). So, the value of binding energy can be used to estimate the ability of a compound to inhibit protein targe [33]. Based on the results, mangrove compounds have the ability to inhibit EGFR as the most suitable target to treat NSCLC. The top three compounds and gefitinib were selected to visualize their interaction.

**Table 1. Molecular docking results**

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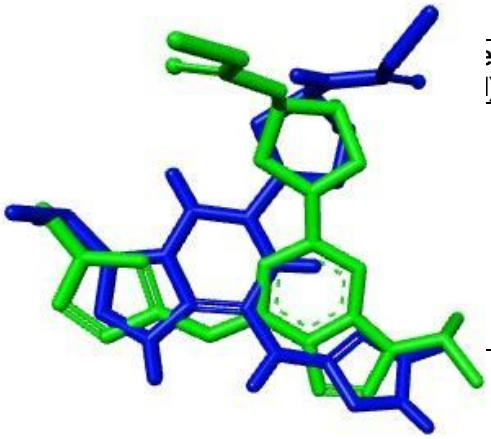

**Fig. 1. Validation of molecular docking, Blue: re-docking results; Green: native ligand. RSMD: 1.91 Å**

### 3.2 Visualization of molecular docking study

The visualization showed in 3D (Fig. 2) and 2D (Fig. 3) form resulted from many amino acid residues of EGFR that bind with the compounds and described in Table 2. Four amino acid residues, Leu718, Leu844, Ala743, and Val726 were found in all interactions between the compounds and EGFR.Previous molecular docking study by Ibrahim et al. revealed that the

active binding sites of EGFR were amino acid residues of Met793, Thr854, Leu718, Leu844, Met766, Val726, Ala743, Lys745, and Met790 [34]. Accordingly, those five amino acid residues were located in the active sites of EGFR. So, all compounds bind in the active site of EGFR. The active site or binding pocket is the binding area of enzyme that involve amino acid residues that play a role in the binding. The interaction of amino acid residues at the active site with the compounds causes compounds to have the ability to inhibit EGFR as a competitive inhibitor. There is a correlation between binding energy and the active sites (binding pocket) of protein target [35].

Stigmasterol and taraxasterol had hydrogen bond with residues of Ser720. Meanwhile, hydrogen bond also found in tretinoin and gefitinib with residues of Lys728. The remaining residues are hydrophobic interactions (Fig. 3). Hydrogen bond and hydrophobic interaction affect the binding energy value. The hydrogen bond is the interaction of hydrogen atoms with electronegative atoms such as fluorine (F), nitrogen (N), and oxygen (O), while hydrophobic interaction is an interaction that occurs between nonpolar molecules which include alkyl-alkyl, pi-alkyl, pi-pi stacked, and pi-pi T-shaped interactions [36,37].

A previous study stated that hydrogen bond and hydrophobic interaction could stabilize the compound when bind in the target protein and change the ΔG value as well as enhance the efficacy of the compound when interacting with the target protein [38]. Similarly, a study revealed that hydrophobic interactions and hydrogen bonds both also make large contributions to compound stability [39]. Thus, hydrogen bond and hydrophobic interaction have a key role in strengthening molecular bond or enhancing binding energy, although it is still debatable between both of them regarding which type has more potential role in increasing the binding energy.

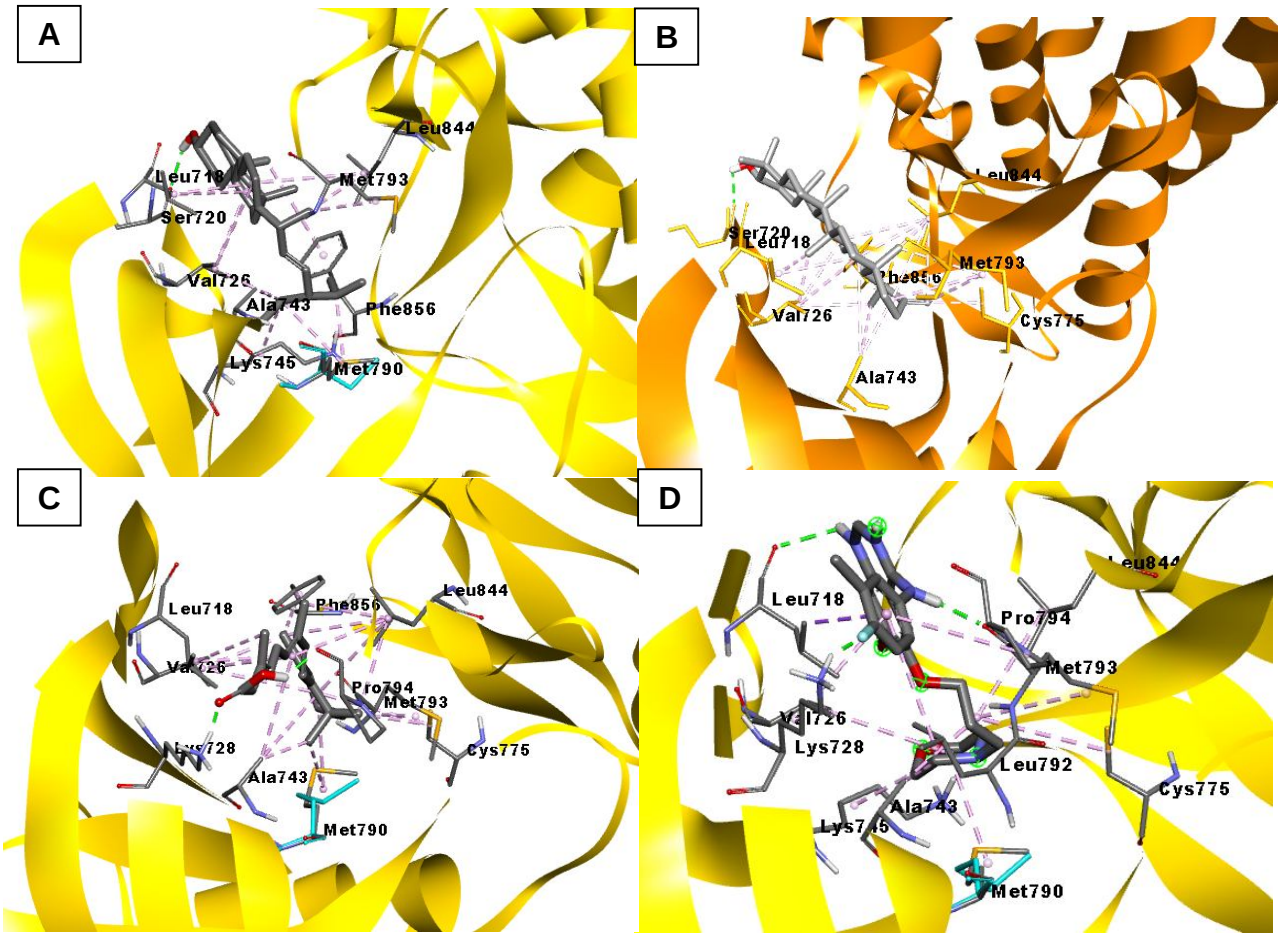


**Fig.2. 3D visualization of molecular docking results between EGFR and the compound A) stigmasterol;B) taraxasterol; C) tretinoin; D) gefitinib.**

**Table 2. The summary of visualization results**

| Compounds | Amino Acid Residues | Molecular Interaction | |
|---|---|---|---|
| | | Hydrophobic Interaction | Hydrogen bond |
| Taraxasterol | Ser720, Leu718(2), Val726(4), Ala743(3), Met793(3), Leu844(5), Cys775, Phe856 | 19 | 1 |
| Stigmasterol | Ser720, Leu718(2), Val726(3), Ala743, Leu844(2), Met793, Met790(2), Lys745, Phe856 | 13 | 1 |
| Tretinoin | Lys728, Pro794, Leu718(2), Val726(3), Ala743(3), Cys775(2), Met790(2), Leu844(5), Met793, Phe856 | 19 | 2 |
| Gefitinib | Lys728(2), Leu718(2), Met793(2), Val726, Ala743(2), Lys745, Cys775, Met790, Leu844, Leu792, Pro794 | 12 | 3 |

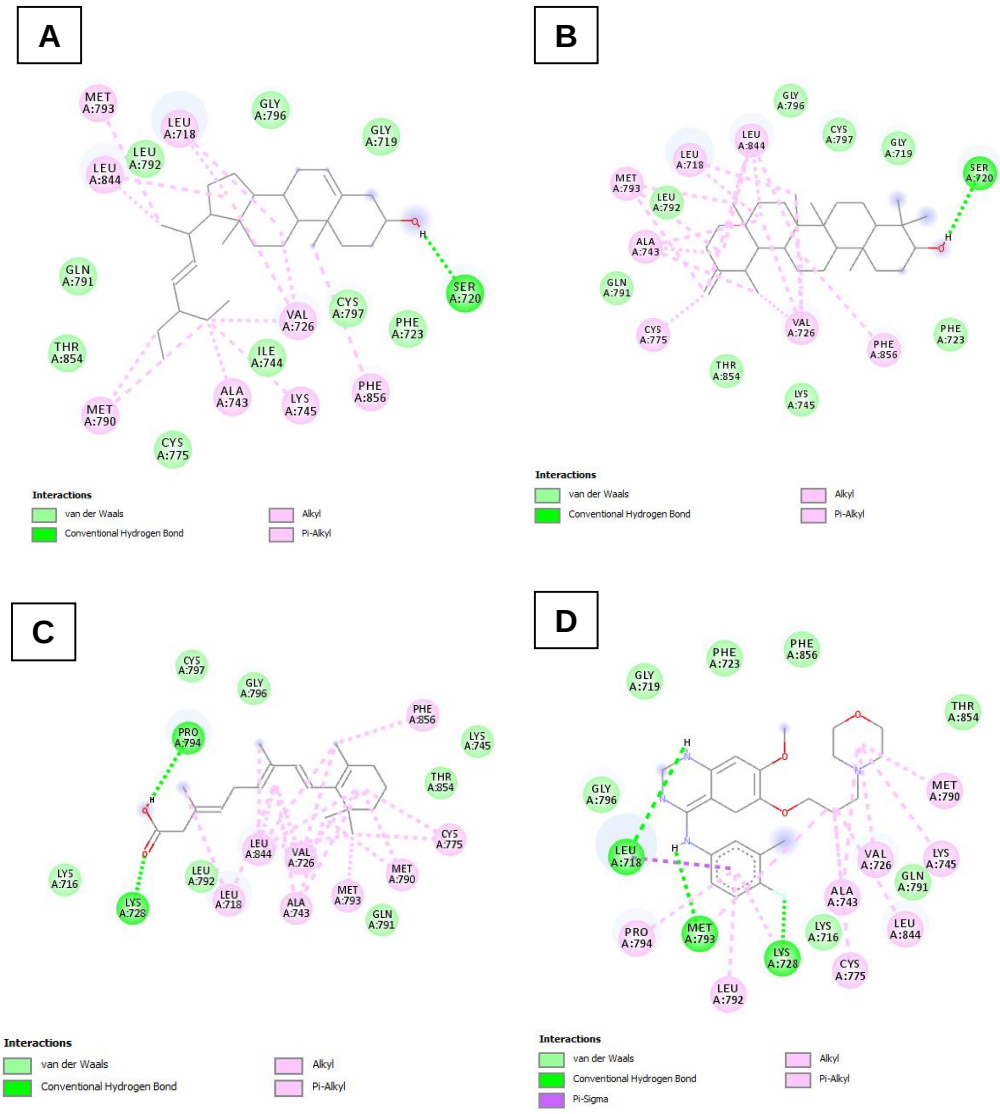


**Fig. 3. 2D visualization of molecular docking results between EGFR and the compound A) stigmasterol;B) taraxasterol; C) tretinoin; D) gefitinib.**

## 3.3 Drug-likeness and Toxicity Analysis

All compounds were found to have no more than one violation (Table 3). Lipinski rules state that for any compound to be considered as a drug-like compound, a compound must obey these criteria: Molecular Weight (MW) <500 Dalton, number of H-bond acceptors <10, number of H-bond donors <5, Log P <5, and if more than one violation were found, then the compound cannot be considered as a drug-like compound [29]. All compounds in this study followed the Lipinski criteria.

On toxicity analysis, most of the compounds are a class of III on acute oral toxicity, which means that based on US EPA classification, the LD 50 values are between 500 mg/kg and

5000 mg/kg [40], while stigmasterol belongs to class I with LD 50 values of less than 50 mg/kg (Table 4). These findings give fundamental data in regards to the toxicological profile of the compounds and might be helpful in choosing the preferred dosage and the route of administration.

| Compound | MW <500 (g/mol) | H-donor | H-acceptor | LogP | LogS | GI absorption | Violation |
|---|---|---|---|---|---|---|---|
| Taraxasterol | 426.72 | 1 | 1 | 4.65 | -8.24 | Low | 1 |
| Stigmasterol | 412.69 | 1 | 1 | 5.08 | -7.46 | Low | 1 |
| Tretinoin | 300.44 | 1 | 2 | 4.28 | -5.34 | High | 1 |
| Heritonin | 258.31 | 0 | 3 | 2.81 | -3.41 | High | 0 |
| Tricin | 330.29 | 3 | 7 | -0.07 | -4.12 | High | 0 |
| Ascochitine | 276.28 | 2 | 5 | 1.84 | -3.06 | High | 0 |

**Table 3. Lipinski results**

| Compound | Carcino-genecity | Eye corrosion | Eye irritation | Ames muta-genesis | Hepato-toxicity | Acute oral toxicity |
|---|---|---|---|---|---|---|
| Taraxasterol | - (0.9571) | - (0.9834) | - (0.8878) | - (0.8400) | - (0.6250) | III (0.8879) |
| Stigmasterol | - (0.8571) | - (0.9886) | - (0.9673) | - (0.8300) | - (0.7750) | I (0.4287) |
| Tricin | - (1.0000) | - (0.9779) | + (0.8092) | - (0.6800) | + (0.7750) | III (0.5920) |
| Heritonin | - (0.9857) | - (0.9799) | - (0.6951) | + (0.5200) | + (0.6250) | III (0.4823) |
| Ascochitine | - (0.8714) | - (0.9852) | - (0.9051) | - (0.8700) | + (0.6750) | III (0.4914) |
| Tretinoin | - (0.6714) | - (0.9886) | - (0.9569) | - (0.7800) | - (0.6000) | III |

**Table 4. Toxicity prediction for taraxasterol, stigmasterol, tricin, heritonin, ascochitine, tretinoin, and gefitinib.**

| | | | | | | |
|---|---|---|---|---|---|---|
| Gefitinib | - (0.9857) | - (0.9886) | - (0.9737) | - (0.5400) | + (0.6750) | (0.8050) III (0.7006) |

*"+" means toxic; "-" means nontoxic. The numbers in brackets indicate the toxicity prediction*

Previous studies reported that taraxasterol and stigmasterol have anti-cancer activities. Bao, et al revealed that taraxasterol inhibited liver cancer cells' growth by inducing cell cycle arrest at G0/G1 phase and apoptosis in vitro and in vivo [41]. In gastric cancer, poor prognosis is associated with overexpression of EGFR. Recent study showed that taraxasterol might play a role as anti-gastric cancer by inactivation of EGFR/AKT1 signaling pathway. It is shown that taraxasterol significantly downregulated EGFR, p-EGFR, AKT1, and p-AKT1 level in the tumor tissues [42]. Another compound, stigmasterol prevents the development of cholangiocarcinoma by downregulating TNF-alpha and VEGFR-2 and suppresses skin cancer by increasing lipid peroxide levels and inducing DNA damage [52]. In the meantime, tricin had proven as anti-cancer. Naoko Seki, et al reported that tricin inhibited proliferation of HSC (Hepatic Stellate Cells) in vitro[43,44,45].

## 4. CONCLUSION

Six compounds have the potential as a drug candidate. Stigmasterol exhibited the highest binding energy. All three compounds bind in the binding pocket of EGFR. All compounds have drug-likeness properties based on Lipinski rules. Moreover, further in vivo and in vitro investigation are needed to bring these compounds at the clinical setting.


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## COMPETING INTERESTS

Authors have declared that no competing interests exist

## AUTHORS' CONTRIBUTIONS

Author HAH, PMA, and T formulated, conceptualized, and designed the research. Author MDR and DA did the program using software, organized the data, and analyzed the result. Author HAH, DA, and RSD wrote the final drafts of the paper, reviewed, and edited the language of the draft. All authors accepted the final draft and are responsible for the manuscript's content and similarity index.

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