



## In Silico Study of Cembranoid-Type Diterpene Targeting Kirsten Rat Sarcoma Virus (KRAS) Gene in Lung Cancer

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### Abstract

Lung cancer is genetic changes in the V-Ki-ras2 gene Kirsten rat sarcoma viral oncogene homolog (KRAS), which is involved in signaling pathways related to proliferation, cell differentiation, and apoptosis. Point mutations in the KRAS gene were detected in 15% to 20% of all Non-Small Cell Lung Carcinoma (NSCLCs) and about 30% of lung adenocarcinomas, with the most common mutation being at codon 12. This study aimed to determine the binding reaction between diterpene-type cembranoid compounds from tobacco leaves (*Nicotiana tabacum L.*) and KRAS in human lung cancer. We found the presence of a cembranoid-type diterpene active compound in the form of thunbergol (C<sub>20</sub>H<sub>34</sub>O) with Gas Chromatography and Mass Spectroscopy (GC-MS) examination. Based on the results of molecular docking, it was found that the diterpene-type cembranoid ligand binds to the KRAS receptor with a yield of G -7.0 kcal/mol, pKi 7.35 M, one hydrogen bond with type ILE36 (1,937 Å). In conclusion, diterpene-type cembranoid can be considered an anticancer compound because of the molecular interaction with cembranoid-type diterpene through cell proliferation and differentiation pathways, as well as apoptosis.

**Keywords:** Cembranoid-type diterpene, in silico, KRAS, lung cancer, mechanism of action.

## Studi In Silico Cembranoid-Type Diterpene Tertaget Gen Kirsten Rat Sarcoma Virus (KRAS) pada Kanker Paru

### Abstrak

Kanker paru adalah perubahan genetik pada gen V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (KRAS) yang terlibat dalam pensinyalan jalur yang berkaitan dengan proliferasi, diferensiasi sel, dan apoptosis. Mutasi titik pada gen KRAS terdeteksi pada 15% hingga 20% dari semua Non-Small Cell Lung Carcinoma (NSCLC) dan sekitar 30% adenokarsinoma paru, dengan mutasi yang paling umum adalah pada kodon 12. Penelitian ini bertujuan untuk mengetahui reaksi pengikatan antara senyawa cembranoid-type diterpene dari daun tembakau (*Nicotiana tabacum L.*) dan KRAS pada kanker paru manusia. Kami menemukan adanya senyawa aktif cembranoid-type diterpene dalam bentuk thunbergol (C<sub>20</sub>H<sub>34</sub>O) dengan pemeriksaan Gas Chromatography and Mass Spectroscopy (GC-MS). Berdasarkan hasil docking molekuler, ditemukan bahwa ligan cembranoid-type diterpene berikatan dengan reseptor KRAS dengan hasil G -7,0 kkal/mol, pKi 7,35 M, 1 ikatan hidrogen dengan tipe ILE36 (1.937 Å). Kesimpulannya, cembranoid-type diterpene dapat dianggap sebagai senyawa anti-kanker karena interaksi molekuler dengan cembranoid-type diterpene melalui jalur proliferasi dan diferensiasi sel serta apoptosis.

**Kata Kunci:** Cembranoid-type diterpene, in silico, KRAS, kanker paru, mekanisme kerja.

## 1. Introduction

Lung cancer is the leading cause of cancer death worldwide—the GLOBOCAN 2018 database, 2.09 million new cases and 1.76 million deaths from lung cancer. The International Agency for Research on Cancer (IARC) released the ten main types of cancer in 2020 that most commonly accounted for more than 60% of cancer cases and 70% of cancer deaths, ranking second was occupied by lung cancer (11.4%)<sup>1</sup>. Lung cancer is the most commonly diagnosed cancer and the leading cause of death from cancer (18.0% of total cancer deaths)<sup>2</sup>.

Histologically, lung cancer can be divided into small-cell and large-cell lung cancer. Large cell lung cancer comprises 80% of lung cancer cases and is subdivided into adenocarcinoma, squamous cell carcinoma, and large cell carcinoma. The development of lung cancer is very complex due to genetic and environmental interactions. Risk factors include tobacco consumption, mainly in the form of smoking, radiation exposure, and environmental toxins, for example, radon, asbestos, arsenic, chromium, nickel, and others<sup>3</sup>. In addition to cigarettes as the leading risk factor, other lung cancer risk factors are environmental exposure to carcinogenic chemicals such as air pollution (burnt smoke and vehicle smoke)<sup>4</sup>.

The effect of tobacco cigarette exposure on normal airway epithelial cell cytology revealed that tobacco smoking causes extensive molecular irregularities in the airway, indicating areas of injury related to pulmonary oncogenesis. The most common symptoms are shortness of breath, coughing (including coughing up blood), and weight loss. The Epidermal Growth Factor Receptor (EGFR), a tyrosine kinase receptor, is often overexpressed in Non-Small Cell Lung Cancer (NSCLC). These receptors play an essential role in the survival of tumor cells<sup>5</sup>.

This expression depends on the histological subtype, most commonly expressed in squamous cell carcinoma but is also often expressed in adenocarcinomas and large cell carcinomas<sup>5</sup>. Molecular changes can be found in the airway epithelium, such

as genetic changes (mutations), copy number variations, and deoxyribonucleic Acid (DNA) methylation<sup>6</sup>.

The most commonly reported gene mutation in lung cancer occurs in the V-Ki-ras2 gene Kirsten rat sarcoma viral oncogene homologous (KRAS). The resulting deviant gene products are involved in signaling pathways associated with proliferation, cell differentiation, and apoptosis<sup>7</sup>. This mutation, especially KRAS, causes cancer to become resistant to standard personal therapies commonly given to cancer survivors, namely tyrosine kinase inhibitors (TKIs) targeted by the individual's genetic makeup, including resistance to conventional chemotherapy and radiation therapies. This conventional method is not specific to cancer cells and attacks normal cells other than existing cancer cells ones. Inadequate treatment and the onset of resistance cause poor prognosis, and the mortality rate of lung cancer patients also increases<sup>8</sup>.

Directs the need to explore Indonesia's biodiversity, which has the potential to be a natural cancer support therapy (adjuvant) that does not cause side effects and is directed directly at cancer target cells. The natural ingredients used in this study are natural ingredients that are known to the public as ingredients that can be the leading cause of lung cancer, but it turns out that it can be the main ingredient supporting lung cancer therapy, namely pure tobacco (*Nicotiana tabacum L*) which is classified as a plantation plant. This plant produces the latest findings and is the subject of scientific discussions that are very interesting to research. Furthermore, it turns out that the most exciting fact after research, this study is the first study of several gene mutases involved in lung cancer by using tobacco as a healing therapy.

Tobacco leaves have various chemical components, many of which are bioactive. Cembranoids are macrocyclic diterpenes that are mainly found in plants belonging to the genus *nicotiana* and pine, as well as marine organisms. Tobacco plants contain the highest content of cembranoid-type diterpene (CBD). The bioactivity of tobacco

CBD is seen in several studies, including having good antifungal, antibacterial, antiviral, and antiparasitic activities and also as a neuroprotective<sup>9,10</sup>. The active ingredient CBD from tobacco plants has also been proven effective as an anticancer agent by inducing apoptosis of cancer cells. Research on CBD from tobacco can lower hepatocarcinoma cell viability<sup>11</sup>. but for lung cancer, adjuvant therapy itself has not been found a supportive reference, especially about the KRAS gene mutation. Through this research, abundant tobacco resources can be utilized, investigating the potential of active compounds of the cembranoid group from pure tobacco leaves (*Nicotiana tabacum L.*) as anticancer through KRAS protein targets and understanding the mechanism of anticancer action so that it can be applied in clinical therapy in the future.

## 2. Methods

This study is an in silico study that aims to determine the interaction between cembranoid-type diterpene group compounds found in tobacco leaves against gene mutations in lung cancer. Several stages must be passed to obtain the desired results, such as sample preparation and maceration, Fourier Transform InfraRed (FTIR) Spectroscopic analysis, Gas Chromatography, Mass Spectroscopy (GC-MS) analysis, and in silico analysis.

### 2.1. Sample Preparation

The first stage is sample preparation and maceration; that is, as much as two thousand five hundred grams of tobacco are identified in the laboratory, then cleaned, washed, thinly sliced, dried, and mashed. Simplisia soaked 96% ethanol (1:10) for three days with several stirrings in dark place storage. They were filtered with vacuum filtration and Whatman filter paper no. 40, evaporated with rotary evaporator T=550C, P=80 mBar.

### 2.2. Analysis of Gas Chromatography and Mass Spectroscopy (GS-MS)

The second stage is the analysis of Gas Chromatography and Mass Spectroscopy

(GC-MS), where tobacco leaf samples are separated first with the GC tool, then identified with the MS tool. This stage must go through various research procedures, such as the search for amino acids that make up the target protein, search for the structure of the active compound of the cembranoid-type diterpene from *Nicotiana tabacum L.*, modeling the 3D structure of proteins, docking and visualization between protein-ligands, and analyzing the interaction of bonds between proteins and ligands.

### 2.3. In Silico Analysis

The essence of this research process is in silico, which is in the process of docking and visualization between protein ligands. Diterpene Cembranoid Ligands are taken from the Web Server Pubchem (<https://pubchem.ncbi.nlm.nih.gov>)<sup>12</sup> database in 3D form. The 3D structure is minimized to obtain the most stable structural conformation using Open Babel software. If the minimization process is complete, right-click on the minimization result, and select convert to ligand pdbqt.

Files that were initially in SDF form will be stored in the form of pdbqt files. The pdbqt file format indicates the presence of a partial charge on each atom. The 3D structure of the Cembranoid Diterpene was obtained by entering the amino acid sequence in the Raptor X program, and the result obtained was data in the form of a PDB file format<sup>13</sup>.

KRAS, ALK, and EGFR preparations were carried out using AutoockTools 1.5.6 by removing water molecules and adding some things such as nonpolar hydrogen, charge, and atoms<sup>14,15</sup>. Then the grid is arranged by creating a grid box that covers the target protein's surface, followed by an autogrid4 program linked to the application.

The process of molecular docking of ligands with KRAS, ALK, and EGFR proteins is carried out with an autogrid4 program. The docking output is in the form of a ligand pose on the active side and its affinity score.

Analysis of docking results was carried out on residues interacting with ligands, Gibbs energy binding ( $\Delta G$ ) parameters, structural

**Table 1.** The result of docking between the receptor and the ligand

Cembranoid-type diterpene	KRAS
$\Delta G$ (Energy Gibbs)	-7.0 kcal/mol
pKi (constraint efficiency)	7,35 $\mu M$
Number of hydrogen bonds	1H $\rightarrow$ ILE36 (1,937 Å)

conformation, affinity, and hydrogen bonds<sup>16</sup> between Diterpene Cembranoids and KRAS, ALK, and EGFR. Visualization of molecular docking results between ligands and proteins was carried out using Edu PyMOL and LIGPLOT software. The visualization uses Edu PyMOL software to clarify the binding side of ligands with proteins. LIGPLOT+ software is used to determine the interaction of the number and distance of hydrogen bonds, hydrophobic interactions, and amino acid residues involved in the interaction between ligands and proteins based on 3D structures<sup>17</sup>. The results of the docking analysis were further visualized using Discovery Studio 4.1, LigPlot+, and LigandScout 3.1 software<sup>18,19</sup>. The interaction between proteins and ligands was analyzed to see the number and type of bonds formed, such as hydrogen bonds, hydrophobic bonds, and van Der Waals bonds<sup>20</sup>.

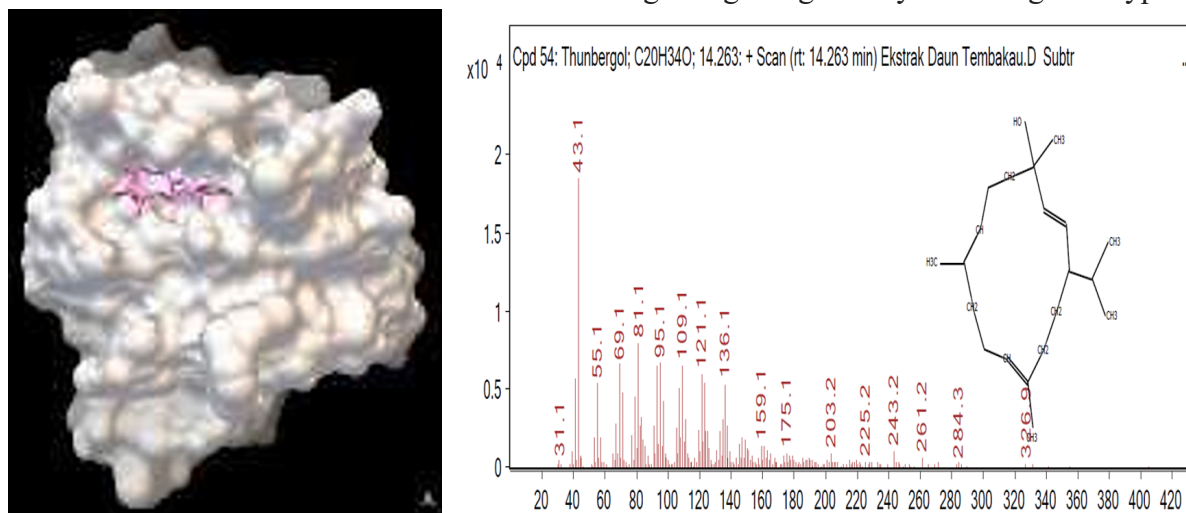
### 3. Result and Discussion

The results obtained from the various processes that have been carried out are very diverse. The active compound cembranoid-type diterpene (KRAS) was obtained in the form of thunbergol in the GC-MS results. Cembratrienol is also called thunbergol or isocembrol. The result can be seen in the

following figure.

After docking, there was an interaction between the Cembranoid-type diterpene (Thunbergol) ligand and the KRAS receptor (Figure 1). To find out which ligands are most likely to interact with a particular receptor can then be based on the prediction of binding free energy. The more negative Gibb's energy, the higher the binding interaction between ligands and receptors. The smaller the resistance value, the stronger the ligand bond to the protein, and the more hydrogen bonds indicate, the stronger the ligand bond to the protein. It can be seen that KRAS has the most negative  $\Delta G$  when interacting with cembranoid-type diterpene ligands. It can be concluded that KRAS has the most significant potential to react with Cembranoid-type diterpenes based on  $\Delta G$  and resistance coefficient values. The following is the result of docking between the receptor and the ligand.

KRAS oncogenic mutations involve point mutations at codons 12 or 13 in exons. KRAS encodes GTPase activity in proteins that regulate cell growth, differentiation, and apoptosis and serves as an EGFR-induced downstream mediator of signaling, chromosomal translocation, and rearrangement at ALK receptors. ALK signaling begins by creating a typical



**Figure 1.** (a) Cembranoid-type diterpene (Thunbergol) and (b) The Interaction Between the Cembranoid-type diterpene (Thunbergol) Ligand and the KRAS Receptor



oncogenic fusion of the ALK gene in the gene leading to constitutive activation of the ALK kinase domain<sup>21</sup>.

This research has benefited the medical world by discovering a CBD pulmonary anticancer docking theory targeted at KRAS, EGFR, and ALK proteins. It has proven to have the opportunity to be developed as one of the considerations of natural plant-based treatment with protein targets, of course, with valid preclinical and clinical trials.

#### 4. Conclusion

This study proves that cembranoid-type diterpene group compounds from tobacco leaves can be used as a targeted pulmonary anticancer for KRAS, EGFR, and ALK proteins, mainly focused on KRAS. Furthermore, this study is consistent with the theory that the most common gene mutation found in lung cancer is in the KRAS gene involving codons 12 or 13.

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#### 6. Conflict of Interest

All authors declared that there was no conflict of interest.

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