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Analyzing the current application status of gefitinib based on the synthetic routes and mechanism of action

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Abstract. This paper focuses on the treatment of lung cancer, which has a high incidence and mortality rate. It highlights the significance of molecularly targeted drugs in anti-tumor therapy and provides an overview of gefitinib, a first-generation inhibitor of epidermal growth factor receptor tyrosine kinase. The article describes the site of action and regulatory mechanism of gefitinib to help understand its pharmacological effects. Three representative synthetic routes of Gefitinib were selected for comprehensive analysis and evaluation, and the optimal route 3 with 4,5-dimethoxy-2-nitrobenzoic acid as the starting raw material and seven steps of demethylation, esterification, side-chain alkylation, reduction, cyclohexylamine, chlorination, and ammonia substitution was chosen by comparing the advantages and disadvantages of the routes. It is the optimal route which is greener and better meets the demand of industrial production. At the same time, this article reviews the effectiveness of gefitinib, its adverse effects, and potential drug resistance. It also highlights the importance of research into the synthetic route of gefitinib, to aid in the design of its production process.

Keywords: Lung Cancer, NSCLC, Gefitinib, Anti-Tumor Agent.

1. Introduction

Located in the chest cavity of the human body, the lungs are vital organs that serve a crucial function in respiration. Through tiny air sacs within the lungs, they facilitate the exchange of gases between oxygen and carbon dioxide. The transformation of a healthy lung cell into an abnormal cancer cell results in the formation of tumors that can cause damage to normal lung tissue. These cancer cells multiply excessively, leading to severe lung cancer that can spread through the bloodstream or lymphatic system, resulting in lesions that can impact individuals' daily lives and threaten their overall well-being. In recent years, the advancement of targeted medication has significantly increased the progression-free survival rates of those diagnosed with lung cancer. Among the first-generation epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKIs), Gefitinib stands out for its ability to precisely target tumor cells, making it an especially effective treatment option for non-small-cell lung cancer patients with EGFR gene mutations. Given its high level of effectiveness, ease of use, and minimal risk of adverse reactions, the development of a reliable synthesis method for Gefitinib holds great potential for application in the field.

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In this paper, the application and synthetic route of gefitinib is discussed. The application part introduces lung cancer and EGFR-TKI, which point out the importance of anti-tumor therapy with targeted drugs, and then introduces gefitinib and explains its mechanism of action in detail. In the synthesis part, three synthetic routes with different starting materials, namely methyl 3-hydroxy-4-methoxybenzoate, 3-hydroxy-4-methoxybenzaldehyde, and 4,5-dimethoxy-2-nitrobenzoic acid, are introduced. Then following a thorough evaluation of multiple factors, such as the accessibility and cost-effectiveness of starting materials, safety and intricacy of the synthesis procedure, route simplicity, ecological impact, and resulting yield, a comparative analysis of various routes is concluded, selecting a route suitable for industrial production, lower cost, and less environmental pollution. At the same time, the current application of Gefitinib is introduced to emphasize the importance of researching its synthetic route.

2. Cancer of Gefitinib

2.1. Cancer

Cancer is a significant global health issue, with a very large number of people dying from malignant tumor diseases worldwide each year, which poses a severe threat to human body health, and morbidity and mortality rates are still on the rise.

In China, the Chinese Journal of Oncology published the 2016 Analysis of Malignant Tumor Prevalence in March 2023, which represents the latest and most comprehensive report on cancer incidence and mortality data in China [1]. According to Figure 1 and Figure 2, the most commonly occurring malignant tumors in China comprise lung cancer, colorectal cancer, gastric cancer, hepatocellular carcinoma, and female breast cancer, among several others. Notably, lung cancer holds the top position among the top 10 malignant tumors in China, irrespective of the frequency of incidences and fatalities.

In January 2021, the Journal of Cancer for Clinicians, a reputable publication under the American Cancer Society, released a report on global cancer statistics for 2020, authored by the IARC team. According to the relevant data, it can be intuitively concluded that the current incidence of female breast cancer is the highest, but despite this, lung cancer is still the most lethal of all cancers [2].

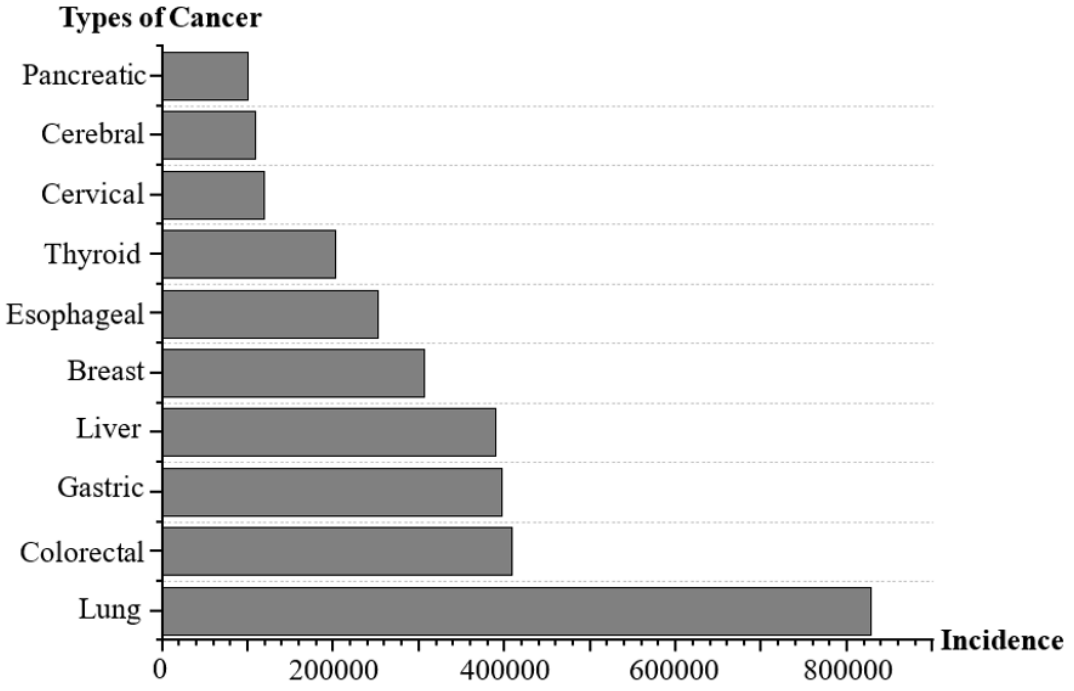

Figure 1. Estimated incidence of the top 10 malignant tumors in China, 2016.

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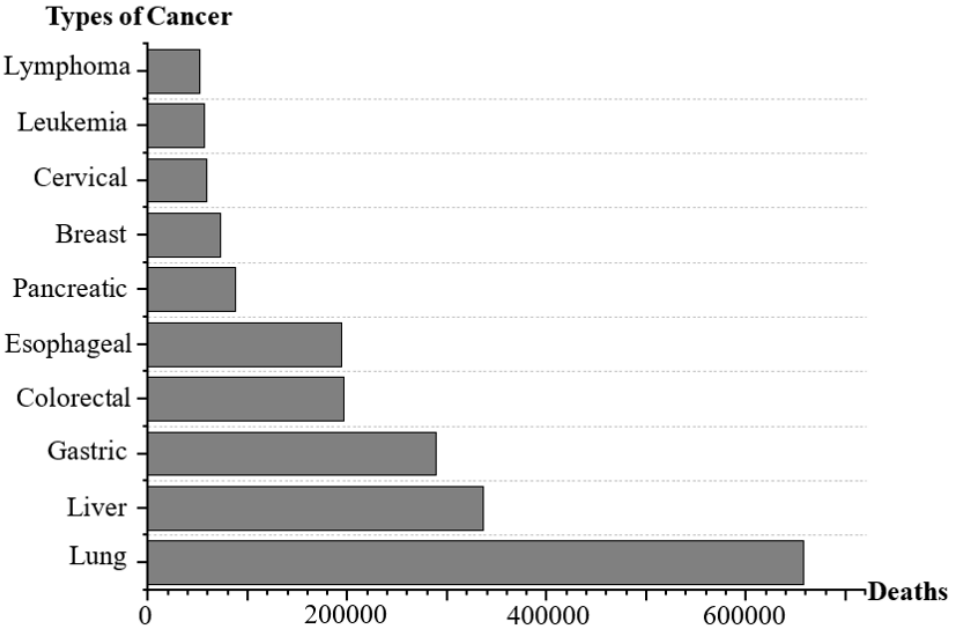

Figure 2. Estimated death of the top 10 malignant tumors in China, 2016.

Lung cancer is commonly classified into two groups based on the type and appearance of cancer cells observed under the microscope. Small cell lung cancer (SCLC) represents approximately 15% of cases, while non-small cell lung cancer (NSCLC) comprises approximately 85% of cases. The onset of lung cancer is a multifaceted procedure in which smoking poses a noteworthy threat. Although nicotine, which is present in cigarettes, doesn't directly initiate cancerous growth, cigarette smoke contains 55 known carcinogens that have been identified by the IARC. Exposure to these carcinogens can trigger modifications in a person's DNA, such as the amplification or deletion of DNA segments or the gain or loss of an entire chromosome [3]. Consequently, smokers face an elevated risk of developing lung cancer. This danger also extends to individuals who are exposed to second-hand smoke, cigars, and e-cigarettes. Except the factors discussed above, other possible factors may increase the risk of lung cancer, such as prolonged exposure to places containing radiation such as rays, a history of chronic lung disease, a family history of genetic predisposition, or emotional depression.

2.2. EGFR-TKI

The EGFR, referred to as the Epidermal Growth Factor Receptor, is a member of the receptor tyrosine kinase family. It comprises three distinct regions, namely the extracellular receptor binding region, the transmembrane region, and the intracellular region that houses the tyrosine kinase domain. Different types of cancer, such as head and neck squamous cell carcinoma, glioblastoma and NSCLC [4]. Abnormal activation of EGFR is closely related to tumour formation, especially in NSCLC. Studies have shown that L858R mutation and exon 19 deletion in the tyrosine kinase domain of EGFR are identified as carcinogenic drivers of NSCLC. When EGFR binds to the corresponding ligand, it will undergo homologous or hetero dimerization, resulting in intracellular tyrosine residue self-phosphorylation, and the activated receptor will recruit the signal complex and continue to activate the downstream signal transduction protein to regulate the growth, invasion, transformation, survival, angiogenesis, and metastasis of tumour cells. Therefore, epidermal growth factor receptor tyrosine kinase inhibitors target EGFR receptors and inhibit EGFR tyrosine kinase activity. The use of EGFR-TKIs is an essential strategy for anti-tumor therapy [5].

EGFR-TKIs can be divided into monoclonal antibodies and small molecule EGFR-TKIs according to their structure and function. Currently, EGFR-TKIs can be categorised into the initial, subsequent, third, and fourth generations [6]. The small EGFR-TKIs compete with ATP for the extracellular ligand-

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binding area of EGFR tyrosine kinase, thus hindering the phosphorylation of tyrosine kinase and obstructing the signaling pathway [7]. AstraZeneca in the UK has successfully developed Gefitinib, the pioneering EGFR-TKIs with exceptional specificity, demonstrating remarkable tolerance and negligible adverse effects. In August 2002, it made its debut in Japan and has since become an essential treatment for NSCLC. In 2005, the product was introduced to the Chinese market and has since become a staple in medical care [8]. The parent structure of the quinazoline ring is similar to the purine ring of ATP, so it can replace ATP, act on the ATP binding site in the EGFR kinase region, occupy the hydrophobic pocket of EGFR, as shown in Figure 2, 1-N binds to the primary amide of the hydrogen bond donor Met793, blocking the interaction between EGFR and ATP. In addition, The 3-chloro-4-fluoraniline substituents in the ATP-binding crack can extend into the target protein's oil-hydrophobic cavity so that gefitinib can better bind to EGFR, thus affecting its downstream signal transduction, inhibiting EGFR tyrosine kinase activity, and playing an anti-tumor role [9].

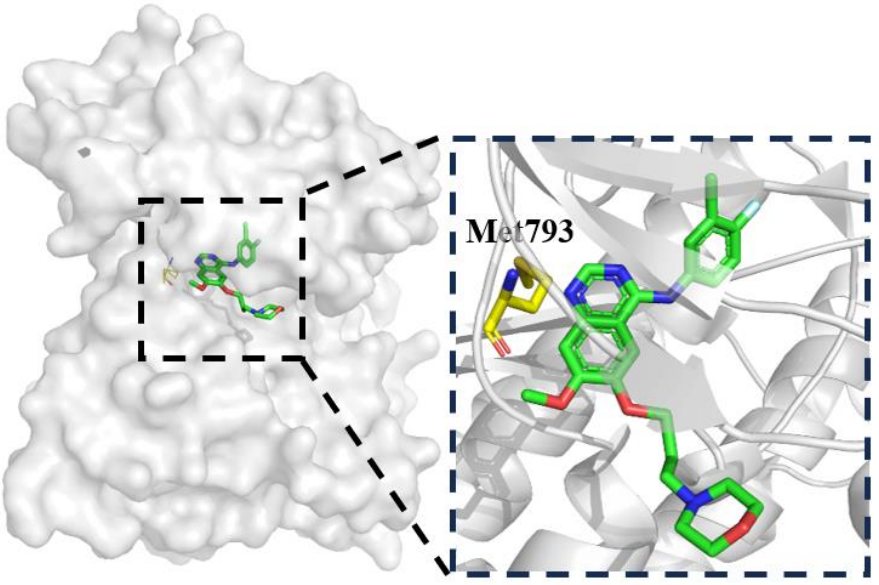

Figure 3. Structure of EGFR and gefitinib complex (PDB ID:2ITY).

3. Synthesis Routes of Gefitinib

3.1. Synthesis Route 1

As Figure 4 shows, Methyl 3-hydroxy-4-methoxybenzoate was used as the starting material for the final synthesis of gefitinib in a total of six steps, including reflux, nitration with nitric acid, reduction, cyclization, chlorination, and amination [10].

The starting material, methyl 3-hydroxy-4-methoxybenzoate, was refluxed with 3-Morpholinopropyl Chloride, then nitrated with nitric acid in a solution formed by acetic acid and acetic anhydride, and the nitro group formed was reduced to an amino group by sodium hypo disulfide in a concentrated hydrochloric acid solvent, then refluxed, cyclized in formamide, immediately followed by dichloro sulfoxide and finally aminated by 3-chloro-4-fluoroaniline to give gefitinib.

The calculated atomic utilization rate is 48.54%. One crucial aspect of this method involves protecting the hydroxyl group with a side chain to prevent any unwanted side reactions. However, purification becomes challenging due to numerous by-products during the nitration process. Moreover, the chlorination reagent employed is a cause for concern as it is not environmentally sustainable, leading to pollution and making industrialization a challenge.

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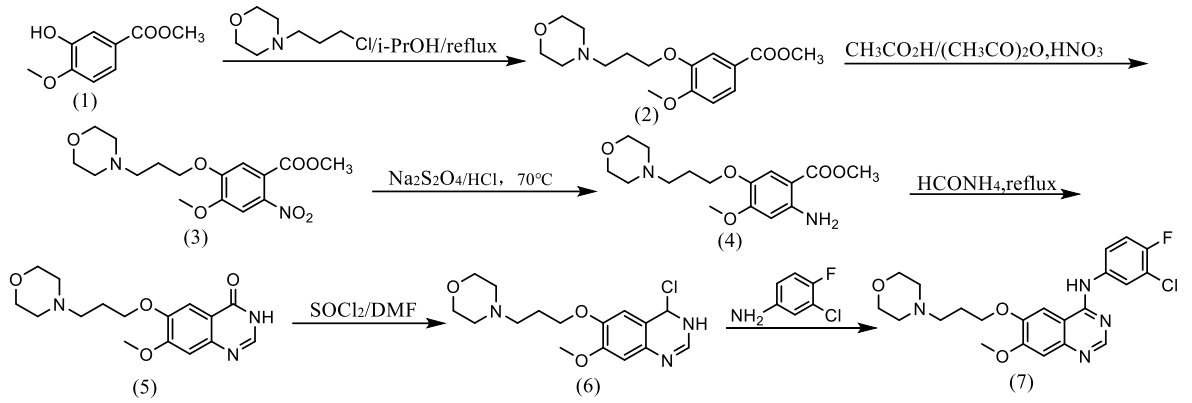

Figure 4. Synthesis Route 1.

3.2. Synthesis Route 2

As shown in Figure 5, starting from 3-hydroxy-4-methoxybenzaldehyde, the intermediate is obtained through reduction condensation and side chain alkoxy oxidation. The intermediate then undergoes direct cyclization with the obtained Schiff base intermediate to synthesize gefitinib [11].

First, 3-hydroxy-4-methoxybenzyl cyanide was prepared by a one-step reaction of raw material and hydroxylamine hydrochloride under acidification (formic acid) conditions. Hydroxylamine hydrochloride first reacts with solvent formic acid to release hydrochloric acid to form light carbamate, and then the light amine generated by dissociation and aldehydes to form oxime reacts with formic acid to esterification and finally produces the product nitrile. In the substitution reaction of phenol hydroxyl hydrogens, potassium carbonate is responsible for binding acids. It creates an alkaline environment that aids in the dissociation of phenolic hydroxyl ions. Meanwhile, potassium iodide acts as an inducer, promoting the dissociation of chlorine ions. Ultimately, nitration on the benzene ring takes place under the condition of nitrosulfur-mixed acid. And the reduction reaction of aromatic nitro with water as a solution, and insurance powder with sodium hydrosulfite as a reducing agent, the nitro is reduced to a mine. Finally, cyan aniline and 3-chloro-4-fluoraniline Schiff base intermediates undergo Dimroth rearrangement under acidic conditions to obtain the final product gefitinib.

This method synthesizes gefitinib directly using a Schiff base ring intermediate, resulting in reduced intermediate production, fewer reaction steps, convenient purification, avoidance of high halogenated hydrocarbons, and reduced pollution. The atomic utilization rate is 49.83%.

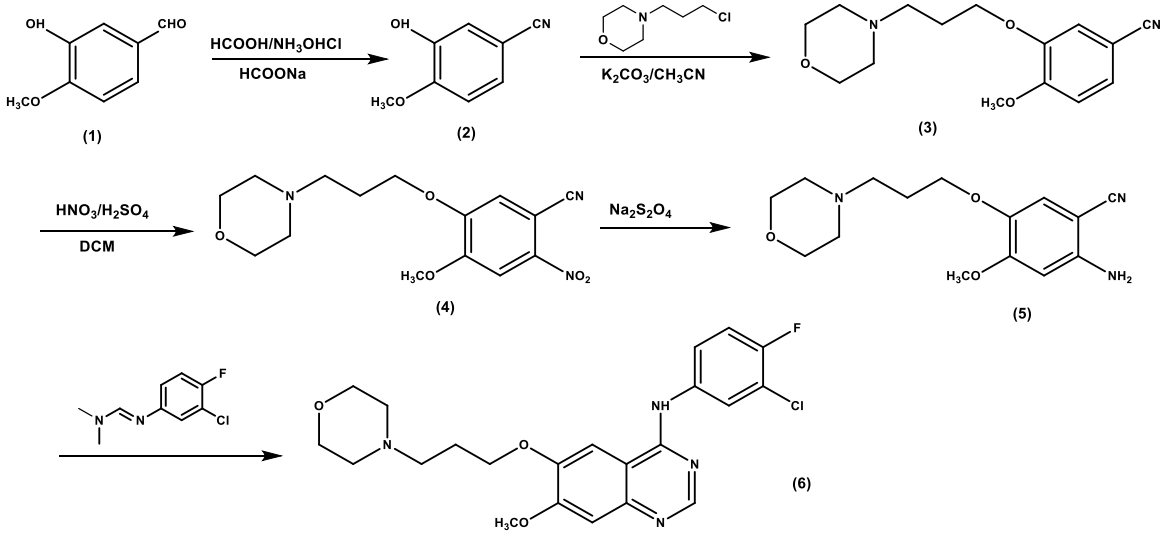

Figure 5. Synthesis Route 2.

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3.3. Synthesis Route 3

As Figure 6 shows, Gefitinib is synthesized through a seven-step process starting with 4,5-dimethoxy-2-nitrobenzoic acid. The process includes various reactions such as demethylation, esterification, side-chain alkylation, reduction, cyclization, chloro- and ammonia substitution [12].

The raw material undergoes demethylation using a potassium hydroxide aqueous solution, replacing L-methionine/methane sulfonic acid with sulfuric acid as the catalyst. Subsequently, potassium carbonate and potassium iodide are also used in the substitution reaction as route 2, with acetonitrile as the solvent. The reduction system consists of iron powder/ammonium chloride, and the cyclization reagent is formamide. Furthermore, phosphorus trichloride is used as the chlorinated reagent. The synthesis of gefitinib is carried out using isopropanol as the reaction solvent, and the amount of 3-chloro-4-fluoroaniline is carefully controlled.

This method not only improves yield, saving costs but also avoids the toxicity of sulfoxide chloride. Besides, it adopts the first connection of the fat side chain to avoid the generation of chlorination by-products in the back. The atom utilization rate is 52.68%, with a total yield of over 50% and a purity of 99.8%, meeting the requirements for industrial production.

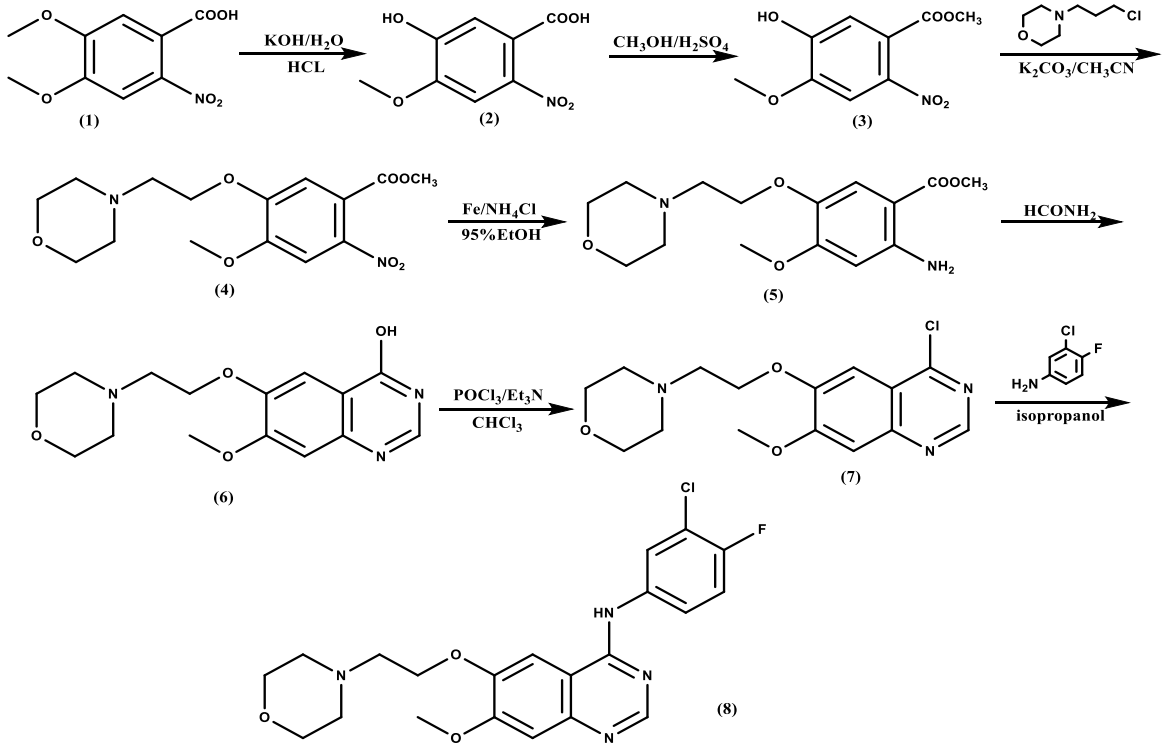

Figure 6. Synthesis Route 3.

3.4. Analyses and Discussions

After comparing and discussing the three routes mentioned above, it has been observed that each of them has its advantages and disadvantages. However, based on a comprehensive analysis of factors such as atomic economy and environmental friendliness, Route 3 is considered the most suitable for industrial production as compared to Route 1 and Route 2. One of the similarities among these routes is that they all protect the hydroxyl group by carrying out the first fat-side chain connection. This helps avoid the generation of by-products during the chlorination reaction, which in turn reduces the damage caused by the conversion of functional groups. As compared to the earlier published synthetic routes that used acetylation and deacetylation for protecting and deprotecting the hydroxyl group, the current process is simpler, more controllable, and generates fewer by-products [13]. This is a common improvement in all three synthetic routes.

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The introduction of gefitinib has given hope to many patients, and researchers have been studying its efficacy and safety in treating NSCLC. According to the FDA, the disease control rate for lung cancer patients in the gefitinib group was 72.41%, which was significantly higher than that of the chemotherapy group. Additionally, the PFS and OSS were also greatly improved [14]. Yukio Hosomi and colleagues conducted a study that showed the OSS of 173 patients who received gefitinib monotherapy was 67%, and the PFS was 11.9 months. The OSS of 172 patients who received gefitinib plus chemotherapy was 84%, and PFS was 20.9 months [15]. This suggests that gefitinib is a viable option for treating NSCLC in the initial stages.

Clinical studies have faced challenges due to gefitinib's negative effects and resistance. Skin toxicity and gastrointestinal reactions are the most common adverse effects but are usually mild and controllable. These side effects can be reduced or eliminated through dose reduction or discontinuation. However, more severe side effects like interstitial pneumonitis, myelosuppression, and hepatotoxicity can be life-threatening if they occur [16].

There are two distinct forms of resistance to gefitinib: intrinsic and acquired. Intrinsic resistance, which happens when the initial treatment with gefitinib fails to produce a response, is connected to the absence of a Kirsten rat sarcoma viral oncogene (KRAS) mutation or a mutation at amino acid 790 in exon 20 of the EGFR (T790M), according to various studies [17, 18]. On the other hand, it's common for patients who initially respond well to develop tumor resistance and experience recurrence at a later stage. This type of resistance is called acquired resistance and it's more prevalent in treating NSCLC patients. Certain patients may not benefit from targeted drug therapy due to somatic mutations in the EGFR gene. These mutations can disrupt genes that rely on the EGFR pathway, trigger alternate pathways, alter signaling channels, impact downstream targets of EGFR, or cause histological changes [19].

Currently, there exist two main strategies to address acquired resistance to gefitinib: either reintroducing the drug after chemotherapy or employing immunotherapy [20, 21]. In clinical practice, it is advised to administer the drug after a meal. This is primarily because high-fat foods have been shown to enhance gefitinib absorption and reduce variability in its effects across patients. Moreover, the postprandial state is deemed more advantageous for assessing bioequivalence in a healthy population [22].

4. Conclusion

Gefitinib is an anti-tumor medication that operates by blocking the tyrosine kinase of the epidermal growth factor receptor. Since 2002, it has been utilised in clinical settings and is well-known for its efficacy, specificity, minimal adverse effects, and general safety. It is the preferred initial treatment for NSCLC. This study revealed that a considerable number of patients who were given gefitinib had EGFR gene mutations, based on an examination of its function and receptors Subsequently, a thorough analysis and comparison of the safety, effectiveness, and convenience of three distinct synthetic pathways was conducted. Finally, a proposed solution was presented to tackle the problem of drug resistance. In recent times, there has been a great deal of exploration into the possible benefits of using gefitinib to treat NSCLC. Meanwhile, the emergence of drug resistance has been found thoroughly. Future studies suggest further exploring the structure and mechanism of gefitinib, finding the cause of side effects, optimising its structure accordingly, and avoiding side effects to the greatest extent. At the same time, attention should be paid to combining gefitinib with other drugs and exploring its therapeutic application in different types of cancer with mutations. Additionally, the synthesis route of gefitinib has the problems of high cost and low efficiency, and many synthesis methods are not environmentally friendly. To optimize production and reduce expenses, it is crucial to identify a more effective and eco-friendly synthesis method.

Authors Contribution

All the authors contributed equally and their names were listed in alphabetical order.

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