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A Novel Synthesis of Anti-Cancer Drug Gefitinib from 6,7-Dimethoxy-3*H*-Quinazolin-4-One

(Suatu Sintesis Baharu Ubat Anti-Kanser Gefitinib daripada 6,7-Dimetoksi-*3H*-Kuinazolin-4-One)

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ABSTRACT

A novel synthesis of the anticancer drug gefitinib (Iressa) through novel alternative pathway has been successfully carried out. The synthesis was performed using 6,7-dimethoxy-3*H*-quinazolin-4-one as the starting material through four reaction stages: Chlorination, nucleophilic aromatic substitution, demethylation, and Williamson etherification to produce gefitinib. The chlorination of 6,7-dimethoxy-*3H*-quinazolin-4-one as the first key step in this synthesis followed by aromatic substitution were effective to produce the target product with high yield (98% for two steps). In addition, synthesis of gefitinib from this precursor omits the necessity for functional group protection and deprotection. Purification was carried out using crystallization and radial chromatography. The structural analysis of the resulting compound was performed using FTIR, ^{1}H-NMR, ^{13}C-NMR, and mass spectrometry. The purity of the resulting compounds was measured using HPLC and melting point measurements (195-197 °C). The overall yield obtained through this pathway was 21%.

Keywords: Anticancer; gefitinib; Iressa; synthesis; 4-chloroquinazoline

ABSTRAK

Suatu sintesis baharu ubat antikanser gefitinib (Iressa) melalui laluan alternatif baharu telah berjaya dijalankan. Sintesis dilakukan menggunakan 6,7-dimetoksi-3H-kuinazolin-4-one sebagai bahan permulaan melalui empat peringkat tindak balas: pengklorinan, penggantian aromatik nukleofilik, demetilasi dan eterifikasi Williamson untuk menghasilkan gefitinib. Pengklorinan 6,7-dimetoksi-3H-kuinazolin-4-one sebagai langkah utama pertama dalam sintesis ini diikuti dengan penggantian aromatik adalah berkesan untuk menghasilkan produk sasaran dengan hasil yang tinggi (98% untuk dua langkah). Di samping itu, sintesis gefitinib daripada prekursor ini menghilangkan keperluan untuk perlindungan dan penyahlindungan kumpulan berfungsi. Pemurnian dijalankan menggunakan penghabluran dan kromatografi jejari. Analisis struktur sebatian yang terhasil dilakukan menggunakan FTIR, 1H-NMR, 13C-NMR dan spektrometri jisim. Ketulenan sebatian yang terhasil diukur menggunakan HPLC dan ukuran takat lebur (195-197 °C). Hasil keseluruhan yang diperoleh melalui laluan ini ialah 21%.

Kata kunci: Antikanser; gefitinib; Iressa; sintesis; 4-klorokuinazolin

INTRODUCTION

Cancer is the second leading cause of death worldwide, following cardiovascular diseases. In 2022, there were 20 million new cancer cases and approximately 9.7 million cancer-related deaths globally. Lung cancer ranks as the leading cause of cancer-related deaths, with 2.5 million cases (12.4% of total cases worldwide) (Bray et al. 2024). Lung cancer can be caused by air pollution from cigarette smoke, motor vehicles, and industrial emissions (Field & Withers 2012; Thandra et al. 2021).

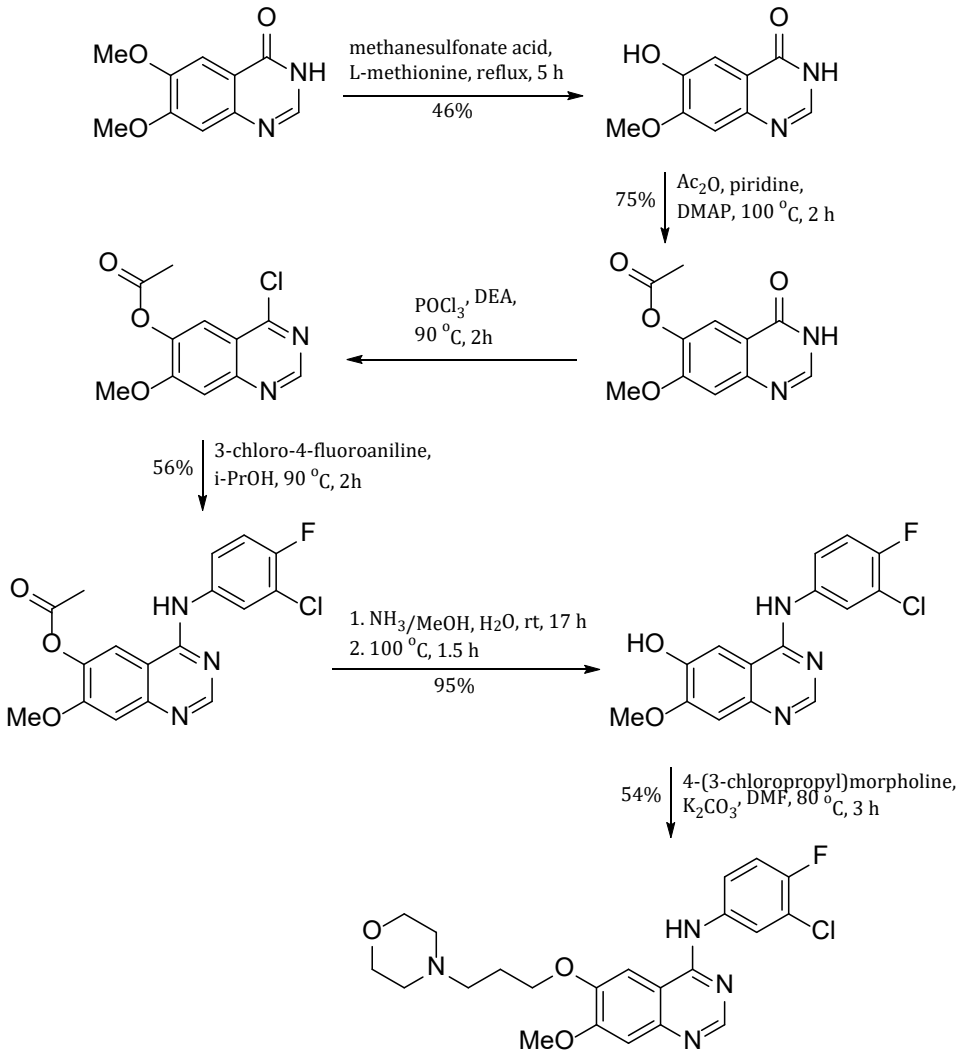
Gefitinib, a first-generation epidermal growth factor receptor tyrosine kinase inhibitor (EGFR-TKI), is one of the current drugs used for lung cancer treatment. It obtained initial approval from the Ministry of Health, Labor, and Welfare (MHLW) and received subsequent approval from the Food and Drug Administration (FDA) in 2015 (Huang, Jiang & Shi 2020). Marketed under the trade name Iressa, gefitinib is manufactured by AstraZeneca. The application of this drug is targeted specifically for Non-Small Cell Lung Cancer (NSCLC) (Mitsudomi et al. 2010; Mok et al. 2009; Paz-Ares et al. 2017; Wu et al. 2017). Gefitinib acts by inhibiting the autophosphorylation process of the tyrosine kinase enzyme on the ATP site of the EGFR tyrosine kinase domain (Da Cunha Santos, Shepherd & Tsao 2011). This

process prevents normal signal transduction through EGFR's internal signaling pathways, leading to a decrease in downstream signal activity that typically triggers cell proliferation and delays apoptosis (Bian et al. 2018).

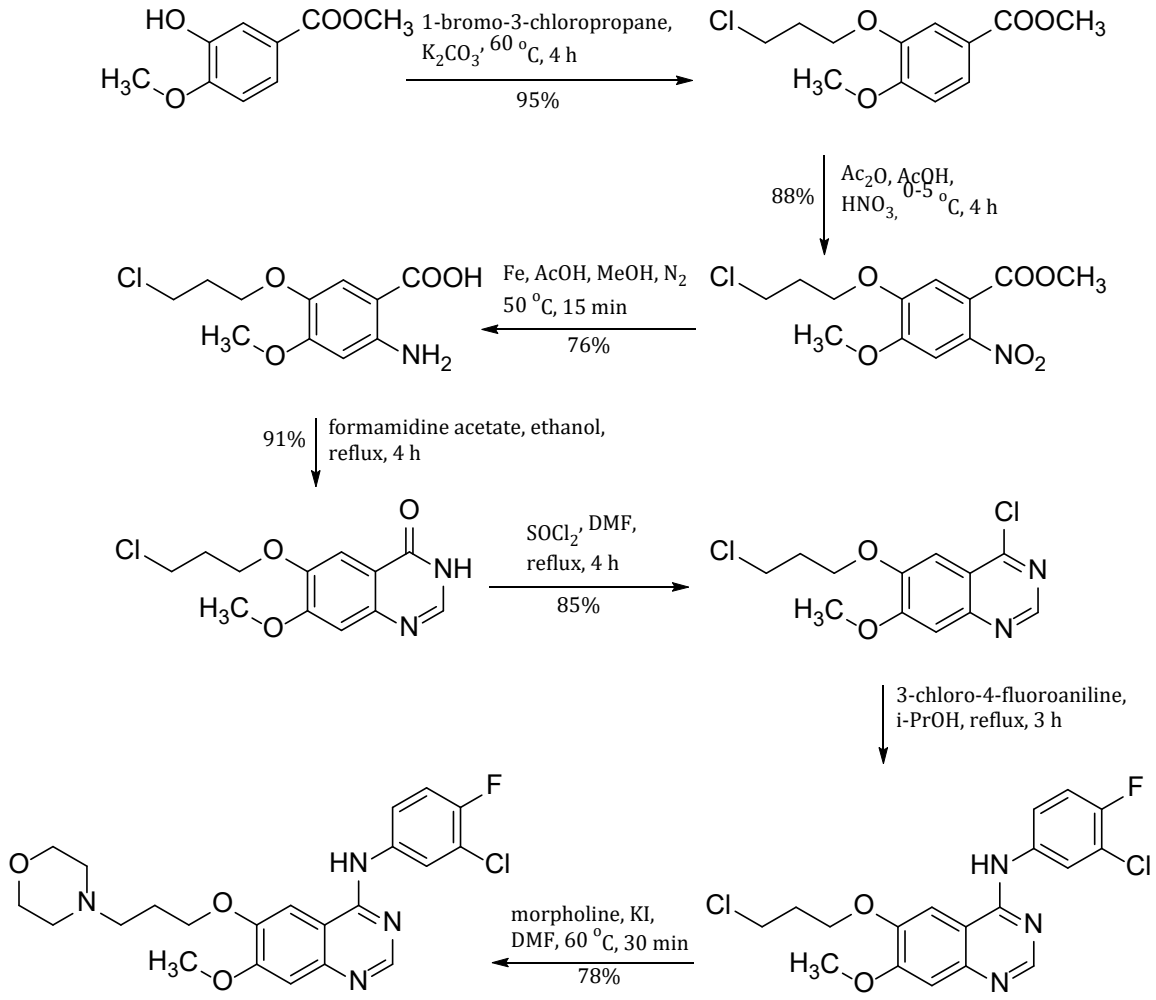
Several studies on the synthesis of gefitinib have been reported (Chandregowda, Rao & Reddy 2007a; Gibson 1998; Maskrey et al. 2019; Ming, You & Ji 2007; Rao, Kankan & Pathi 2013). Gefitinib was first synthesized by Gibson in 1987 and employed by AstraZeneca for industrial-scale gefitinib production (Gibson 1998). In this method, gefitinib was synthesized through six reaction steps. The reactions commenced with demethylation of 6,7-dimethoxy-*3H*-quinazolin-4-one, followed by acetylation, halogenation, aromatic amine nucleophilic substitution, deacetylation, and O-alkylation (SCHEME 1). The total yield of this method is 10%.

Ming, You and Ji (2007) synthesized gefitinib from methyl 3-hydroxy-4-methoxybenzoate as presented in Scheme 2. This route was chosen to avoid demethylation reactions resulting in relatively low yields. The synthesis commenced with O-alkylation of 3-hydroxy-4-methoxybenzoate, followed by nitration, reduction of nitro group, cyclization, chlorination, aromatic amine nucleophilic substitution, and substitution of Cl atom by morpholine (Scheme 2). The total yield via this route was greater compared to the Gibson method, namely, 36%.

Given the two synthetic pathways for gefitinib, it requires 6 to 7 steps to obtain gefitinib. The longer the synthetic pathway, the more materials, solvents, energy, and waste are produced. This does not align with the 12 principles of green chemistry, particularly principle 1 (prioritize waste prevention rather than its treatment and reuse), principle 4 (preserve efficacy of function while reducing toxicity of designed chemicals), principle 6 (minimize energy requirements), and principle 8 (reduce/avoid unnecessary derivatization (protection/deprotection)) (Rosa et al. 2022; Sheldon 2012).



SCHEME 1. Gibson (1998) synthesis of gefitinib



SCHEME 2. Ming, You and Ji (2007) synthesis of gefitinib

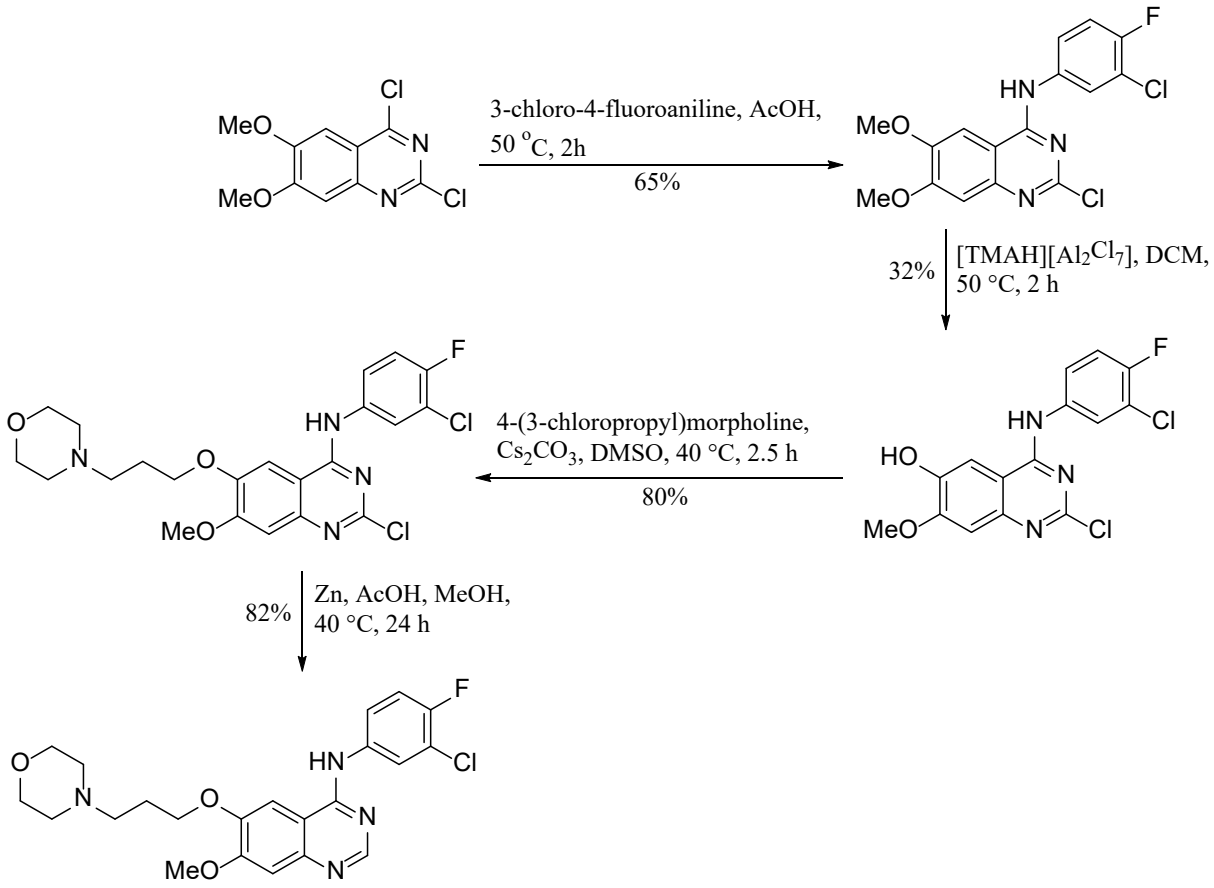
Efforts to shorten the reaction steps in the synthesis of gefitinib were undertaken by Maskrey et al. (2019). The synthesis was carried out through four reaction steps. The first step involved nucleophilic substitution of aromatic amine with Cl atom at C-4 position on the 2,4-dichloro-6,7-dimethoxyquinazoline as the starting material. The next step was demethylation on the methoxy group followed by O-alkylation at C-6 position and dechlorination at C-2 position on the quinazoline ring (Scheme 3). This pathway was relatively short with a reasonably high total yield of 14%. However, its workup process is impractical as it requires multiple extraction techniques and the addition of several additives such as LiCl and 2-mercaptonicotinic acid. Thirdly, the relatively expensive starting material used, dichloroquinazoline, poses a drawback.

This study aims to synthesize gefitinib through a simpler pathway. It is expected that this pathway will make the synthesis more effective by minimizing the number of derivatives and waste produced, reducing the energy and materials used during the synthesis process, and increasing the overall yield.

MATERIALS AND METHODS

MATERIALS AND INSTRUMENTATIONS

Compound 6,7-dimethoxy-3*H*-quinazolin-4-one was purchased from TCI. 3-chloro-4-fluoroaniline, methanesulfonic acid, L-methionine, 1-bromo-3-chloropropane, morpholine, 4-(3-chloropropyl) morpholine, NaOH, anhydrous K_2CO_3, and anhydrous Na_2SO_4 were purchased from Sigma-Aldrich. The solvent used includes isopropanol, dimethyl formamide (DMF), and methanol were purchased from Smart Lab Indonesia. Substrate 2-5 were synthesized as described herewith. All work-up and purification procedures were carried out with reagent-grade solvents. Analytical thin-layer chromatography (TLC) was performed using TLC Silica Gel 60 F254. Radial chromatography was performed using Silica Gel 60 PF254 (7733). Fourier Transform Infrared (FTIR) spectra were obtained on Shimadzu IR prestige-21 Japan. Nuclear magnetic resonance (NMR) spectra were recorded on an Agilent DD2 (^{1}H 500 MHz, ^{13}C 126 MHz)



SCHEME 3. Maskrey et al. (2019) synthesis of gefitinib

spectrometer with the references of TMS (0.00 ppm) and $CDCl_3$ (77.16 ppm). High-resolution mass spectrometry (HRMS) spectra were obtained by Electrospray Ionization-Time of Flight (ESI-TOF) Waters LCT Premier XE mass spectrometry. Purity analysis using Shimadzu LC-20AT High-Performance Liquid Chromatography (HPLC). The melting points of compounds 2-5 were measured using Stuart® protected by BioCote®.

SYNTHESIS OF 4-CHLORO-6,7-DIMETHOXYQUINAZOLINE (**2**)

The compound 6,7-dimethoxy-3*H*-quinazolin-4-one (**1**) (10 g; 0.05 mol) was placed into a 100 mL two-neck flask, followed by the addition of 50 mL of thionyl chloride ($SOCl_2$) and 2 mL of dimethylformamide (DMF) dropwise, with stirring until the compound 8 dissolved. The mixture was then refluxed for 4 h. After the reaction was complete, the remaining $SOCl_2$ was evaporated using a rotary evaporator. Subsequently, the resulting solid was dissolved in 100 mL of chloroform ($CHCl_3$), washed with 2 × 150 mL of saturated sodium bicarbonate ($NaHCO_3$) solution, and 2 × 150 mL of distilled water. The organic phase was separated and dried over anhydrous sodium sulfate (Na_2SO_4). The solution obtained was filtered and evaporated using a rotary evaporator. The resulting product was purified by recrystallization using ethyl acetate. The obtained product **2** (9.83 g, 90% yield) exists in the form of a white solid with mp of 185-186 °C. Based on HPLC analysis, the product purity was determined to be 99.9%. The product was analyzed using FTIR, NMR, and mass spectrometers. The product is known, and the spectroscopic properties are consistent with the data available in the literature (Yadav et al. 2016).

Compound **2**, IR (KBr): υ (cm^{-1}): 2974 (C-H Ar), 2835 (CH_3-O), 1616, 1565 & 1513 (C=C Ar), 1348 (C-N Ar), 1234 (Ar-O). ^{1}H-NMR ($CDCl_3$, 500 MHz), δ (ppm): 8.85 (H-2, s, 1H), 7.36 (H-5, s, 1H), 7.31 (H-8, s, 1H), 4.06 (7-OCH_3, s, 3H), 4.05 (6-OCH_3, s, 3H). ^{13}C-NMR ($CDCl_3$, 125 Hz), δ (ppm): 158.9 (C-7), 156.6 (C-4), 152.4 (C-2), 151.3 (C-6), 148.9 (C-9), 119.4 (C-10), 106.8 (C-8), 102.5 (C-5), 56.5 (7-OCH_3), 56.3 (6-OCH_3). HRMS (ESI TOF) calcd for $C_{10}H_{10}ClN_2O_2$ $[M+H]^+$ 225.0425, found 225.0427.

SYNTHESIS OF N-(3-CHLORO-4-FLUOROPHENYL)-6,7-DIMETHOXYQUINAZOLIN-4-AMINE (**3**)

Compound 4-chloro-6,7-dimethoxyquinazoline (**2**) (6.30 g; 0.03 mol; 1 equiv) was introduced into a two-necked 250 mL flask, followed by the addition of 3-chloro-4-

fluoroaniline (10.00 g; 0.069 mol; 2.3 equiv) and 100 mL isopropanol. The mixture was stirred at room temperature for 1 h. The resulting precipitate was filtered, washed with isopropanol, and then dried in the oven at 60 °C for 24 h. The obtained product **3** (9.65 g, 98% yield) exists in the form of a white solid with mp of 253-255 °C. Based on HPLC analysis, the product purity was determined to be 99.4%. The product was analyzed using FTIR, NMR, and mass spectrometers. The product is known, and the spectroscopic properties are consistent with the data available in the literature (Waiker et al. 2014).

Compound **3**, IR (KBr): υ (cm^{-1}): 3429 (N-H Ar), 3029 (C-H Ar), 2830 (CH_3-O), 1634, 1580 & 1500 (C=C Ar), 1354 (C-N Ar), 1221 (Ar-O). ^{1}H-NMR (DMSO-d_6, 500 MHz), δ (ppm): 11.54 (4-NH, s, 1H), 8.88 (H-2, s, 1H), 8.35 (H-8, s, 1H), 8.03 (H-6', dd, 1H, J = 6.9; 2.8 Hz), 7.75 (H-2', m, 1H), 7.55 (H-5', t, 1H, J = 9 Hz), 7.34 (H-5, s, 1H), 4.02 (6-OCH_3, s, 3H), 3.99 (7-OCH_3, s, 3H). ^{13}C-NMR (DMSO-d_6, 125 Hz), δ (ppm): 158.6 (C-4), 156.9 (C-7), 155.5 (C-4', d, $J_{C\text{-}F}$ = 246,4 Hz), 150.7 (C-6), 149.4 (C-2), 134.7 (C-1'), 134.7 (C-9), 127.1 (C-2'), 125.8 (C-6', $J_{C\text{-}F}$ = 7,1 Hz), 119.6 (C-3'), 117.4 (C-5', $J_{C\text{-}F}$ = 21,9 Hz), 107.8 (C-10), 104.4 (C-5), 100.4 (C-8), 57.5 (C-7), 56.9 (C-6). HRMS (ESI TOF) calcd for $C_{16}H_{14}ClFN_3O_2$ $[M+H]^+$ 334.0753, found 334.0759.

SYNTHESIS 4-((3-CHLORO-4-FLUOROPHENYL)AMINO)-7-METHOXYQUINAZOLIN-6-OL (**4**)

The compound **3** (1.00 g; 0.003 mol; 1 equiv) was placed in a two-necked 50 mL flask, followed by the addition of L-methionine (0.895 g; 0.006 mol; 2 equiv) and 10 mL of methanesulfonic acid. The mixture was stirred and heated at 120 °C for 2 h. Subsequently, the mixture was cooled to room temperature, then added to 100 mL of ice water and stirred. The mixture was then treated with a solid NaOH until reaching pH = 4. The resulting solid was filtered using a Buchner funnel, washed with water, and then dried in a desiccator for 24 h. The solid was then washed by placing it in 10 mL of methanol, stirred, and heated to 80 °C to remove impurities. The resulting solid was filtered, dried, and rechecked using TLC. The obtained product **4** (0.976 g, 93% yield), in the form of a white solid with mp of 280-283 °C. Based on HPLC analysis, the product purity was determined to be 91.7%. The product was analyzed using FTIR, NMR, and mass spectrometers. The product is known, and the spectroscopic properties are consistent with the data available in the literature (Gibson 1998).

Compound **4**, IR (KBr): υ (cm^{-1}): 3375 (O-H Ar), 2937 (C-H Ar), 2830 (CH_3-O), 1620, 1580 & 1500 (C=C Ar), 1421 (C-N Ar), 1221 (Ar-O). ^{1}H-NMR (DMSO-d_6, 500 MHz), δ (ppm): 11.15 (6-OH, s, 1H), 10.59 (4-NH, s, 1H), 8.84 (H-2, s, 1H), 8.05 (H-5, s, 1H), 8.01 (H-2', dd, 1H, J = 6.8; 2.6 Hz), 7,70 (H-6', ddd, J = 8,9; 4,3; 2,6), 7.53 (H-5', t, 1H, J = 9.1 Hz), 7.41 (H-8, s, 1H), 4.01 (7-OCH_3, s, 3H); ^{13}C NMR (DMSO-d_6, 125 Hz), δ (ppm): 158.5 (C-4), 156.6 (C-7), 155,5 (C-4', d, $J_{C\text{-}F}$ = 245,8 Hz), 149.0 (C-6), 148.6 (C-2), 135.1 (C-9), 134,8 (C-1', d, $J_{C\text{-}F}$ = 3,4 Hz), 126.9 (C-2'), 125,6 (C-6', d, $J_{C\text{-}F}$ = 7,5 Hz), 119.6 (C-3'), 117,3 (C-5', d, $J_{C\text{-}F}$ = 21,9 Hz), 108.3 (C-10), 107.4 (C-5), 100.3 (C-8), 57.0 (7-OCH_3). HRMS (ESI TOF) calcd for $C_{15}H_{12}ClFN_3O_2$ $[M+H]^+$ 320.0597, found 320.0597.

SYNTHESIS OF GEFITINIB (**5**)

A total of 100 mg of compound **4** (0.238 mmol; 1 equiv) and 65.7 mg (0.475 mmol; 2 equiv) of K_2CO_3 were introduced into a carousel tube, followed by the addition of 1.1 mL of DMF. The mixture was then stirred at 80 °C for 1 h. To the mixture, 77.8 mg of 4-(3-chloropropyl)morpholine (0.475 mmol; 2 equiv) was added and stirred for 3 h. Subsequently, the mixture was cooled to room temperature, and 5 mL of cold water was added. The resulting mixture was extracted with 2 × 5 mL of chloroform. The chloroform filtrate was dried with anhydrous Na_2SO_4. The resulting filtrate was subjected to TLC and concentrated, followed by purification through radial chromatography. The resulting product of gefitinib **5** (33 mg, 25% yield) is in the form of white solid with mp of 195-197 °C. Based on HPLC analysis, the product purity was determined to be 99.9%. The product was analyzed using FTIR, NMR, and mass spectrometers. The product is known, and the spectroscopic properties are consistent with the data available in the literature (Chandregowda, Rao & Reddy 2007a).

Gefitinib (**5**), IR (KBr): υ (cm^{-1}): 3362 (N-H Ar), 2950 (C-H Ar), 2815 (CH_3-O), 1621, 1580 & 1500 (C=C Ar), 1421 (C-N Ar), 1221 (Ar-O). ^{1}H NMR (DMSO-d_6, 500 MHz), δ (ppm): 9.55 (4-NH, s, 1H), 8.49 (H-2, s, 1H), 8.12 (H-6', dd, 1H, J = 6.9; 2.8 Hz), 7.80 (H-5, s, 1H), 7.44 (H-5', t, 1H , J = 10 Hz), 4.18 (H-1", t, 2H, J = 5 Hz), 3.93 (7-OCH_3, s, 3H), 3.58 (H-3a, t, 4H, J = 5 Hz), 2.47-2.46 (H-3", m, 2H), 2.39 (H-2a, br s, 4H), 2.02-1.97 (H-2", m, 2H). ^{13}C NMR (DMSO-d_6, 125 Hz), δ (ppm): 156.5 (C-4), 154.6 (C-7), 154.0 (C-4', d, $J_{C\text{-}F}$ = 241.2 Hz), 152.7 (C-6), 148.8 (C-2), 147.4 (C-9), 137.3 (C-1', d, $J_{C\text{-}F}$ = 3 Hz), 124.0 (C-2'), 122.8 (C-6', d, $J_{C\text{-}F}$ = 6.8 Hz), 119.2 (C-3', d, $J_{C\text{-}F}$ = 18,5 Hz), 117.0 (C-5', d, $J_{C\text{-}F}$ = 21,6 Hz), 109.2 (C-10), 107.8 (C-5), 103.0 (C-8), 67.6 (C-1"), 66.6 (C-3a), 56.3 (7-OCH_3), 55.4 (C-2a), 53.9 (C-3"), 26.3 (C-2"). HRMS (ESI TOF) calcd for $C_{22}H_{23}ClFN_4O_3$ $[M-H]^-$ 445.1448, found 445.1443.

RESULTS AND DISCUSSIONS

In this study, the synthesis of gefitinib commenced with a stable 6,7-dimethoxy-*3H*-quinazolin-4-one as the starting material. Chlorination of this compound, followed by nucleophilic aromatic substitution using 3-chloro-4-fluoroaniline, offers selective substitution at C-4 position with high yield when reacting with fluoroaniline (98%). The synthesis was completed in 4 steps.

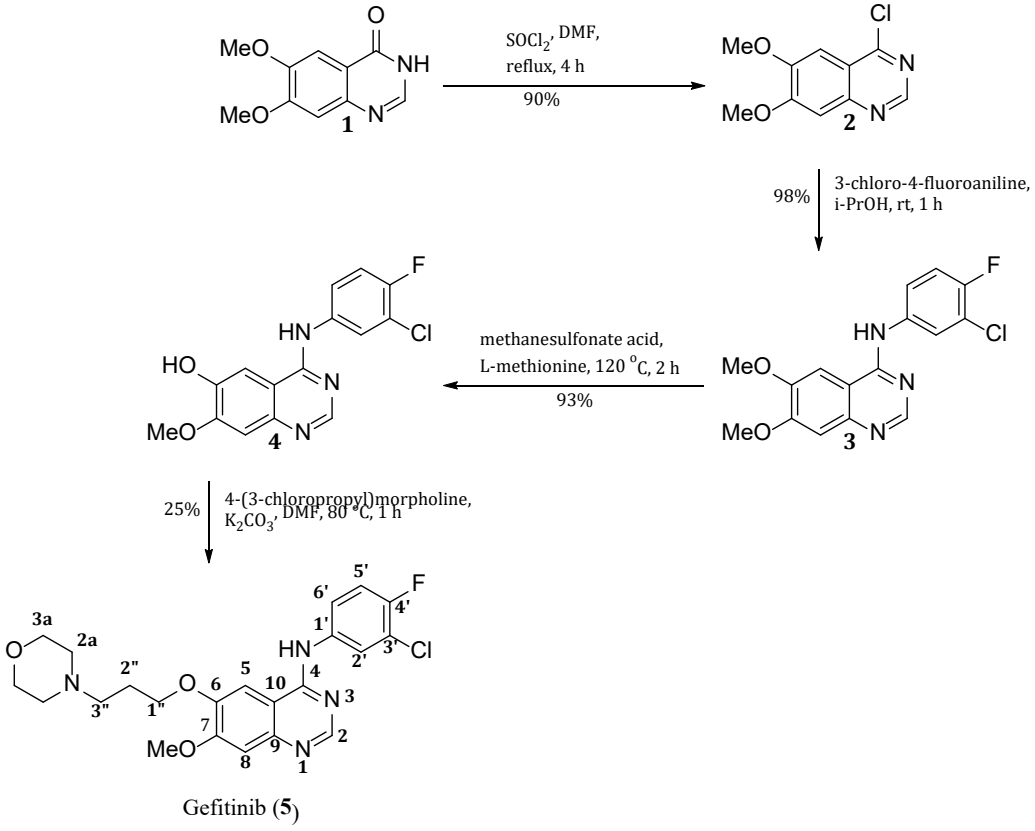
Previous reports accessed Gefitinib from 2,4-dichloro-6,7-dimethoxyquinazoline, where two chlorine groups

were present at the C-2 and C-4 positions which lead to moderate to good yield of 65% probably due to the competitive reaction with additional chlorine group presents when reacting with fluoroaniline. Therefore, this study represents an improvement over the previously reported work by Maskrey et al. (2019). In general, this pathway offered an alternative pathway to the existing routes to access gefitinib with fewer steps and higher overall yields. The absence of protection and deprotection groups contributes to the greener process of our proposed route.

The synthesis was carried out through four stages of reactions: Chlorination, aromatic nucleophilic substitution, demethylation, and Williamson etherification (Scheme 4). Using FTIR, HRMS, ^{1}H, and ^{13}C NMR, the synthesized intermediates and final products were characterized. Intermediates and final products compound **2-5** were identified from FTIR by their characteristics peaks at 2963-2817, 1620-1500, and 1234-1221 cm^{-1}, indicating the existence of aromatic C-H, C=C-C, ether C-O stretching vibration, respectively (Firdaus et al. 2022; Hamed et al. 2024; Nandiyanto, Oktiani & Ragadhita 2019).

TABLE 1. Reported methods for the synthesis of gefitinib

	Gibson (1998)	Ming, You & Ji (2007)	Maskrey et al. (2019)	This report
Overall yields	10%	36%	14%	21%
Steps	6	7	4	4
Key features	*O*-demethylation of 6,7-dimethoxy-3*H*-quinazolin-4-one	Formation of quinazolin ring from methyl 3-hydroxy-4-methoxy-benzoate	Application of ionic liquids for demethylation, followed by dehalogenation	Chlorination of 6,7-dimethoxy-*3H*-quinazolin-4-one, followed by aromatic nucleophilic substitution



SCHEME 4. Synthesis of gefitinib

The synthesis begins with the chlorination reaction of 6,7-dimethoxy-*3H*-quinazolin-4-one (**1**) using $SOCl_2$, resulting in the compound 4-chloro-6,7-dimethoxyquinazoline (**2**). Compound **2** is an intermediate classified as a chloroquinazoline. This compound is one of the intermediates commonly found in the synthesis pathway of gefitinib (**5**) (Chandregowda, Rao & Reddy 2007a; Gibson 1998; Ming, You & Ji 2007; Rao, Kankan & Pathi 2013) and various FDA-approved drugs (Boobalan et al. 2017; Erickson et al. 2014; Kovacevic et al. 2018; Mao et al. 2020; Wang et al. 2021; Wei et al. 2019). The resulting product is a white solid (yield 90%).

The ^{1}H NMR spectrum of compound **2** shows five hydrogen atoms in different environments. The methyl protons appear at δ_H 4.05 (3H, s) and 4.06 (3H, s). The three aromatic protons appear at δ_H 7.31 (1H, s), 7.36 (1H, s), and 8.85 (1H, s). The ^{13}C NMR spectrum also matches the number of carbon atoms in compound **2**, showing 10 carbon atoms in different environments. The molecular mass of compound **2** was further confirmed by HRMS (ESI TOF). The HRMS (ESI TOF) spectrum of compound **2** shows that the $[M+H]^+$ ion has an m/z value of 225.0427, consistent with the molecular formula $C_{10}H_{10}ClN_2O_2$ (calculated m/z = 225.0425).

The second step is the nucleophilic aromatic substitution between compound **2** and 3-chloro-4-fluoroaniline in isopropanol solvent, resulting in the formation of N-(3-chloro-4-fluorophenyl)-6,7-dimethoxyquinazolin-4-amine (**3**). The Cl atom attached to C-4 in compound **2** is a good leaving group and is easily replaced by nucleophiles such as the $-NH_2$ group in 3-chloro-4-fluoroaniline. Similar reactions involving chloroquinoline compounds with 3-chloro-4-fluoroaniline have been widely reported and yield relatively high yields (Chandregowda, Venkateswara Rao & Chandrasekara Reddy 2007b; Rahman, Korashy & Kassem 2014). The resulting compound **3** in this study is a white solid with a yield of 98%. The reaction between compound **2** and 3-chloro-4-fluoroaniline is proposed to proceed via an S_N2 mechanism.

The ^{1}H-NMR spectrum of compound **3** shows nine hydrogen signals in different environments. The hydrogen signal at δ_H 11.54 (1H, s) indicates the presence of an -NH group. This suggests that the substitution of Cl in compound **2** with the amine group from 3-chloro-4-fluoroaniline has occurred. The additional aromatic hydrogen signals at δ_H 7.55 (1H, t, J = 10 Hz), 7.75 (1H, s), and 8.03 (1H, d, J = 5 Hz) indicate that the resulting product is compound **3**. The molecular mass of compound **3** was further confirmed by HRMS (ESI TOF). The HRMS (ESI TOF) spectrum of compound **3** shows that the $[M+H]^+$ ion has an m/z of 334.0759, which is consistent with the molecular formula $C_{16}H_{13}ClFN_3O_2$ (calculated m/z [M+H]+ = 334.0750).

The third step involves the demethylation of compound **3** using L-methionine in methanesulfonic acid, resulting in the formation of 4-((3-chloro-4-fluorophenyl)amino)-7-methoxyquinazolin-6-ol (**4**). L-methionine is a type of thiol that has been used as a nucleophile to cleave aryl methyl ethers via an S_N2 reaction. L-methionine is also a relatively new large-scale reagent used to convert alkyl aryl ethers into phenols. The alkylated L-methionine, which is the final product, is a non-volatile and non-genotoxic vitamin (Fujii, Irie & Yajima 1977). The resulting product is a white solid with a yield of 93%.

The ^{1}H-NMR spectrum of compound **4** shows nine hydrogen atom signals in different environments. The appearance of hydrogen signals at δH 4.01 (3H, s) and 10.59 (1H, s) indicates that one methoxy group ($-OCH_3$) in compound **3** has been converted into a hydroxyl group (-OH). The ^{13}C-NMR spectrum data reveal that there are 15 carbon atoms in the product, consistent with the number of carbon atoms in compound **4**. The HMBC of 2D NMR spectrometer was used to observe long-range correlations (2 or 3 bonds) between H and C atoms. This HMBC data was used to confirm that the demethylated $-OCH_3$ group is located at the C-6 position. The spectrum of HMBC can be seen in Figure 1. Based on the figure, it can be seen that the H atom **a** of the $-OCH_3$ group at C-7 correlates with the C-7 atom (**h**), the H atom **b** at C-8 correlates with C-6 (**g**), C-7 (**h**), C-9 (**f**), and C-10 (**e**), and the H atom **c** at C-5 correlates with C-4 (**i**), C-6 (**g**), C-7 (**h**), and C-9 (**f**). The molecular mass of compound **4** was subsequently confirmed with HRMS (ESI TOF). The HRMS (ESI TOF) spectrum of compound **4** shows that the $[M+H]^+$ ion has an m/z of 320.0595, consistent with the molecular formula $C_{15}H_{12}ClFN_3O_2$ (calculated m/z $[M+H]^+$ = 320.0602).

The fourth step is the synthesis of gefitinib (**5**) from compound **4** using 4-(3-chloropropyl)morpholine and K_2CO_3 in DMF solvent. In this stage, two compounds are produced simultaneously: gefitinib (**5**) and a gefitinib analog (**6**) (Figure 2). These compounds were separated by radial chromatography, yielding 31% and 12%, respectively. Telliez et al. (2007) reported that compound **6** is a specific ITK-EGFR and a potent inducer of apoptosis in PC3 prostate cancer cells, hence the serendipity of obtaining this compound in the reaction could open a pathway to access this moiety through optimization of reaction conditions.

Furthermore, the structure of compound **5** was confirmed using ^{1}H-NMR, ^{13}C-NMR, and mass spectrometry. The ^{1}H-NMR spectrum shows thirteen signals of hydrogen atom in different environments. The appearance of five additional proton signals at δ_H 2.02-1.96 (m, J = 6.6 Hz, 2H), 4.18 (t, J = 6.35 Hz, 2H), 2.47 (t, J = 7 Hz, 2H), 2.39 (br s, 4H), 3.58 (t, J = 4.55, 4H) indicates the protons from the 4-(3-chloropropyl)morpholine moiety substituted at the hydroxyl group at C-6. The ^{13}C-NMR spectrum data show twenty carbon atom signals in different environments, consistent with the number of carbon atoms in gefitinib. The molecular mass of compound **5** was further confirmed using HRMS (ESI TOF). The HRMS (ESI TOF) spectrum of compound **5** shows that the $[M-H]^-$ ion has an m/z of 445.1443, which is consistent with the molecular

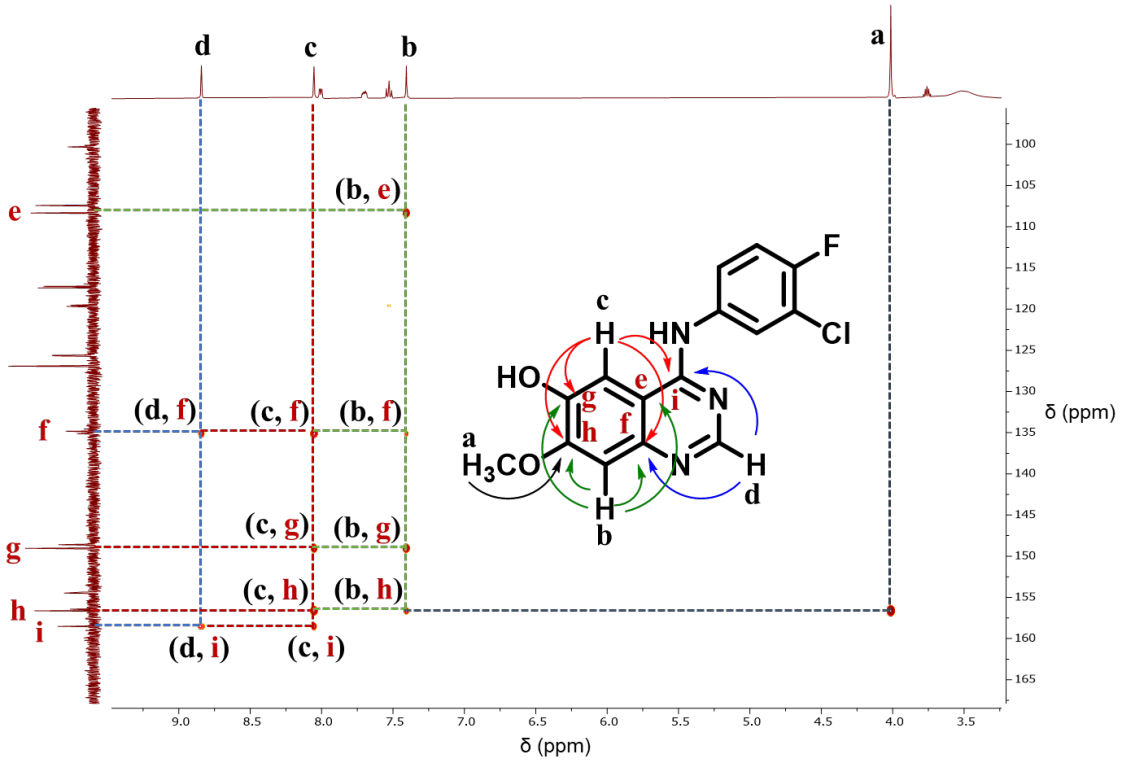


FIGURE 1. HMBC analysis of compound **4**

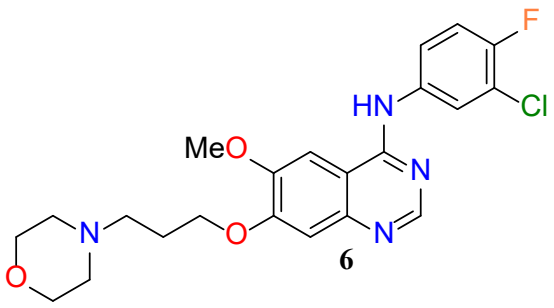


FIGURE 2. Structure of gefitinib analog

formula for $C_{22}H_{23}ClFN_4O_3$ (calculated m/z = 445.1443). The results of this study offer a more effective synthesis pathway for gefitinib. With fewer steps, this pathway can reduce the amount of intermediates and waste generated, improve the efficiency of energy required, and increase the total yield obtained.

CONCLUSION

In conclusion, the synthesis of gefitinib was successfully accomplished in four steps, starting from 6,7-dimethoxy-*3H*-quinazolin-4-one with 21% overall yields. The key reactions in the synthesis were chlorination of the precursor followed by aromatic nucleophilic substitution, which showed yields improvement from previous reports upon reacting with fluoroaniline. Additionally, starting from 6,7-dimethoxy-*3H*-quinazolin-4-one omits the necessity for functional group protection and deprotection.

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