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PREPARATION OF ERLOTINIB HYDROCHLORIDE

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ABSTRACT

Erlotinib hydrochloride (Tarceva) is a targeted cancer therapy drug approved for treatment of non-small cell lung cancer (NSCLC), pancreatic cancer and several other types of cancer. An efficient and economical synthetic method of title compound from ethyl 3,4-dihydroxybenzoate is described in 6 steps with overall yield of 51 %.

Keywords: Erlotinib, non small cell lung cancer, synthesis.

1. INTRODUCTION

Erlotinib was discovered in the early 1990s as a selective inhibitor of the epidermal growth factor receptor (EGFR, IC_{50} = 2 nm) by binding specifically to ATP of EGFR tyrosine kinase intracellular [1]. The EGFR overexpression often leads to the formation and growth of solid tumors, especially in the lungs, including 40 – 80 % of non-small cell lung cancer cases. It involves the development of tumors, proliferation, apoptosis, metastasis, and angiogenesis [2]. EGFR also protects cells from the malignant tumor cytotoxic effects of chemotherapy and radiotherapy, which makes therapeutic effect of this approach significantly reduced.

Erlotinib (Figure 1) is approved by US FDA for the treatment of locally advanced or metastatic non-small cell lung cancer that has failed at least one prior chemotherapy regimen. For pancreatic cancer, erlotinib is used in combination with gemcitabine in all stages [3]. Studies have shown that the using erlotinib in combination with chemotherapy can increase survival rates to over 19 % [4]. As a moleculary targeted cancer therapy drug, erlotinib has few side effects and helps to relieve pain and cough, so improves the quality of patient life.

We reported herein an efficient and economical approach for the preparation of erlotinib hydrochloride from the readily available starting materials in Vietnam, which can be scale up to provide drug raw materials for Vietnam Pharmaceutical Industry.

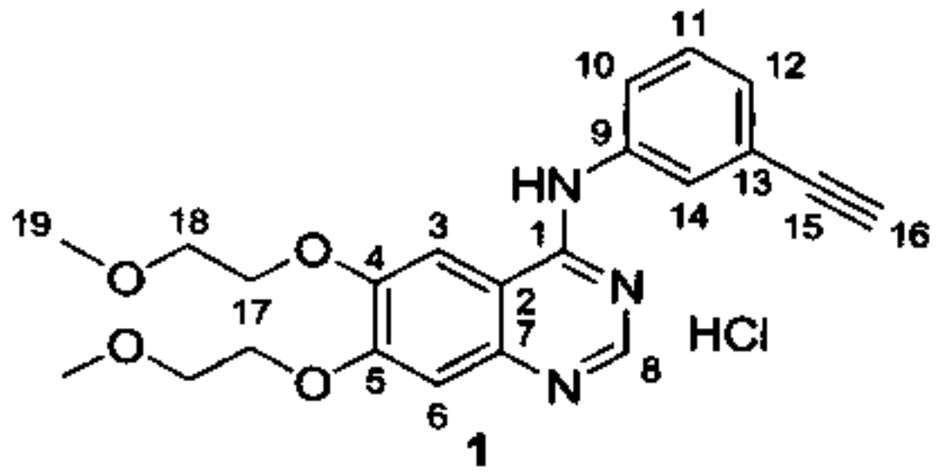

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Figure 1. Chemical structure of erlotinib hydrochloride (**1**).

2. EXPERIMENTAL

All reagents and solvents were purchased from Sigma-Aldrich, Acros and Merck and used withoutpre-purification. Silica gel for column chromatography (0.040 – 0.063 mm) and thin layer chromatographywere purchased from Merck (Germany). NMR spectra were recorded on a Bruker Avance 500 (Germany) spectrometer using TMS as internal standard. Chemical shifts were reported in parts per million (ppm)downfield from internal tetramethylsilane (TMS). MS spectra and HPLC were recorded on aLC_MS Agilent 1100 (USA). All reactionswere monitored by thin layer chromatography (TLC) using silica gel 60 coated plates F254 (aluminum sheets). Visualization was performed by UV at 254 and 354 nm.

Ethyl 3,4-bis(2-methoxyethoxy)benzoate (3): A mixture ofethyl 3,4-dihydroxybenzoate (1.0 g; 5.5 mmol) (**2**), K_2CO_3 (1.82 g, 13.0 mmol) in DMF (10 mL) was heated and stirred at 100 °C for 1 h. After cooling of the reactional mixture to 50 °C, 1-methoxy-2-bromoethane (1 mL) was addedandheatedat 80 °C for 2 h.The resulted mixture was cooled to room temperature, filtredand washedthe solid with ethyl acetate(10 mL). Organic layers were combined, driedover Na_2SO_4 and concentrated under reduced pressure to afford a yellow solid (**3**, 94.3 %). **^{1}H-NMR** (500 MHz, $CDCl_3$)δ (ppm): 1.37 (t, 3H, CH_3-CH_2, J = 7.0 Hz), 3.45 (s, 3H, CH_3O), 3.46 (s, 3H, CH_3O),3.80 (m, 4H, 2x CH_3OCH_2), 4.20 (m, 4H, 2x $CH_3OCH_2CH_2O$), 4.34 (q, 2H, CH_3CH_2, J = 7.0 Hz),6.90 (d, 1H, J = 8.5 Hz, H-5), 7.58 (d, 1H, J = 2.0 Hz, H-2),7.66 (dd, 1H, J = 8.5 & 2.0 Hz, H-6).

Ethyl 4,5-bis(2-methoxyethoxy)-2-nitrobenzoate (4): HNO_3conc.(4.5 mL) was added drop wise to a solution of **3**(500 mg, 2.0 mmol) in CH_3COOH (1.9 mL)at 0 – 5 °C and stirred at room temperature for 24 h.The mixture was poured in to the ice water and ethyl acetate was added. Organic layer was washed with saturated $NaHCO_3$ solution, dried over $Na_2SO_4$and concentrated under reduced pressure to yield a yellow liquid (90.7 %).**^{1}H-NMR** (500 MHz, $CDCl_3$)δ (ppm): 1.34 (t, 3H, CH_3CH_2, J = 7.0 Hz), 3.44 (s, 6H, 2xOCH_3), 3.79 (m, 4H, 2xCH_3OCH_2), 4.24 (m, 4H, 2x CH_2O), 4.36 (q, 2H, CH_2CH_3, J = 7.0 Hz), 7.12 (s, 1H, H-3), 7.50 (s, 1H, H-6).

Ethyl 2-amino-4,5-bis(2-methoxyethoxy)benzoate (5): propan-2-ol (1.59 mL) was added in to a flask containing Pd/C 10 % (45 mg). Solution of ammonium formate (0.265 g) in water (0.2 mL)was added to the flask and the mixture was stirred at room temperature for 1 min to activate Pd.Then **4** (0.15 g, 0.41 mmol) was added to the reactional mixture and continue to stir at room temperature for 20 min. The resulted mixture was filtred and washed with ethyl acetate and propan-2-ol. The filtrate was concentrated under reduced pressure to give a residue which was extracted with ethyl acetate, dried over Na_2SO_4, to obtain **5**(95.1 %). **^{1}H-NMR** (500 MHz, $CDCl_3$)δ (ppm): 1.36 (t, 3H, CH_3, J = 7.0 Hz), 3.44 (2s, 6H, 2xCH_3),

3.72 &3.77 (2t, 2x2H, 2xOCH_2, J = 5.0 Hz), 4.09 & 4.14 (2t, 2x2H, $2CH_2O$, J = 5.0 Hz), 4.30 (q, 2H, CH_2CH_3, J = 7.0 Hz), 6.30 (s, 1H, H-6), 7.46 (s, 1H, H-3).**^{13}C-NMR**(125 MHz, $CDCl_3$) δ (ppm): 166.9 (C=O), 150.1 (C-5), 141.2 (C-7), 133.6 (C-4), 116.5 (C-3), 108.8 (C-2), 101.2 (C-6), 77.3 (C-18, C-18'), 74.1 (C-17, C-17'), 60.9 (C-19, C-19'), 52.5 (CH_2-CH_3), 18.5 (CH_2-CH_3).

6,7-bis(2-methoxyethoxy)-4-quinazolone (6): Amine **5** (0.55 g, 1.75 mmol) and $HCOONH_4$ (0.11 g, 1.75 mmol) were disolved in H_2N-CHO (0.83 mL). The mixture was stirred and heated at 160 °C for 1,5 h under inert atmosphere. Cold water was added and forming precipitates were filtred, washed by cold water and dried. The filtrate was extracted with chloroform and the organic layer was washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. The precipitates and chloroform residue were combined, treated with diethyl ether and filtred to afforded **6** (79.6 %). **^{1}H-NMR** (500 MHz, $CDCl_3$)δ (ppm): 3.50 (s, 6H, 2 xOCH_3), 3.87 (m, 4H, 2xOCH_2), 4.30 (m, 4H, 2xCH_2O), 7.17 (s, 1H, H-6), 7.62 (s, 1H, H-3),8.04 (s, 1H, H-8), 11.72 (s, 1H, NH). **^{13}C-NMR** (125 MHz, $CDCl_3$)δ (ppm): 162.3 (C=O), 155.0 (C-5), 148.9 (C-8), 145.4 (C-7), 142.3 (C-4),115.9 (C-3), 109.4 (C-2), 106.8 (C-6), 70.7 & 70.5 (C-18, C-18'), 68.7 & 68.6 (C-17, C-17'), 5 9.3(C-19, C-19'). **ESI-MS: $[M+H]^+$**295.11.

4-chloro-6,7-bis(2-methoxyethoxy)quinazolin (7): Dimethylammoniumpyridine (42 mg, 0.34 mmol) and $POCl_3$ (0.32 mL) were added to a flask containing quinazolone 6 (0.1 g, 0.34 mmol) in tolunene (5 mL) at room temperature. The mixture was heated at 90 °C for 20 min then maintained at 85 °C for 30 min. When the reaction finished (monitored by TLC), $POCl_3$ was eliminated by evaporator under reduced pressure. The residue was diluted with toluene (3×50 mL), poured in to ice water and extracted with ethyl acetate (4×50 mL). Organic layers were combined, dried over Na_2SO_4, evaporated of solvents to result a white solide (90.3 %).**^{1}H-NMR** (500 MHz, $CDCl_3$) δ (ppm): 3.49 & 3.50 (2s, 2x3H, 2xOCH_3), 3.88-3.90 (m, 4H, OCH_2), 4.34-4.38 (m, 4H, CH_2O), 7.47 (s, 1H, H-3), 7,51 (s,1H, H-6), 8.9 (s, 1H, H-8). **^{13}C-NMR**(125 MHz, $CDCl_3$) δ (ppm): 159.8 (C-5), 157.0 (C-1), 151.6 (C-8), 151.4 (C-4), 147.7 (C-7), 119.7 (C-2), 106.9 (C-6), 104.3 (C-3), 70.6 &70.3 (C-18, C-18'), 69.1 & 69.0 (C-17, C-17'), 59.4 (C-19, C-19').

Erlotinib hydrochloride (1) : Quinazoline **7** (0.17 g, 0.54 mmol) in H_2O (2.61 mL) and 3-ethynylaniline (0.07 mL) were added in to a flask. The mixture was heated at 30 °C and a solution HCl conc. (0.05 mL) was added. The mixture was maintained at 40 °C for 1 h. After cooling, the solid was separated by filtration, washed with water and ether, then dried at 40°C to obtain a white solid **1**(87.5 %). **^{1}H-NMR** (500 MHz,DMSO-d6) δ (ppm): 3.35 & 3.36 (2s, 2x3H, 2xOCH_3), 3.74 - 3.79 (m, 4H, 2xOCH_2), 4.18 (s, 1H, ≡CH), 4.28 - 4.32 (m, 4H, 2xCH_2O), 7.20 (d, 1H, J = 8,0 Hz, H-12),7.23 (s, 1H, H-6), 7,40 (t, 1H, J = 8.0 Hz, H-11), 7.90 - 7.92 (m, 2H, H-10, H-3), 8.01 (s, 1H, H-14),8.49 (s, 1H, H-8),9.8 (br, 1H, NH). **^{13}C-NMR** (125 MHz, DMSO-d6) δ (ppm):156.2 (C-1), 153.6 (C-5), 152.7 (C-8), 148.1 (C-4), 147.0 (C-7), 139.9 (C-9), 128.8 (C-11), 126.2 (C-12), 124.8 (C-14), 122.6 (C-10), 121.6 (C-13), 109.0 (C-2), 108.2 (C-6), 103.5 (C-3), 83.5 (C-15), 80.4 (C-16), 70.1 & 70.0 (C-18, C-18'), 68.0 (C-17, C-17'), 58.3 (C-19, C-19'). **ESI-MS $[M+H]^+$** 394.34 (tính toán 394.44).

3. RESULTS AND DISCUSSION

The reported synthesis methods of erlotinib often start from3,4-hydroxy-benzaldehyde, 3,4-hydroxybenzoic acid or ethyl 3,4-hydroxybenzoate [5 - 9]. But by using the staring material

as aldehyde we have received the low yield and the product purification was difficult due to the many byproducts. By combining the method of Reddy [6], Knesl [7] and Barghi [9], we have prepared erlotinib hydrochloride according to the process including six steps from the ethyl 3-4-hydroxy benzoate (Figure 2).

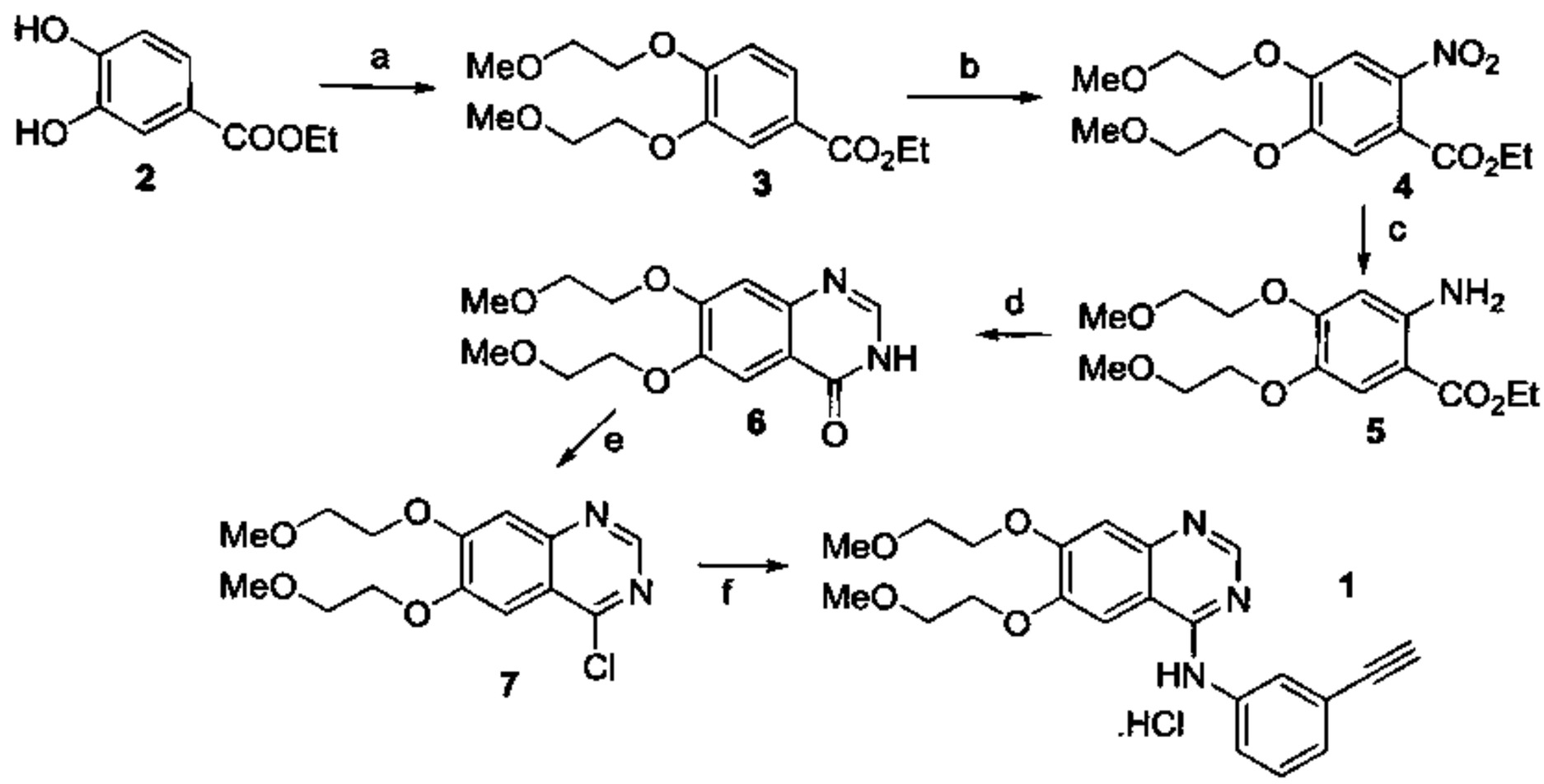


Figure2. Synthetic pathway of erlotinib hydrochloride (1): a) $CH_3OCH_2CH_2Br$, K_2CO_3, DMF, 100 °C, 2h. b) CH_3COOH, HNO_3, 5 °C, 30 min. c) propanol-2, ammonium formate, Pd/C, 25 ^{0}C. d) ammonium formate, formamide, 160 °C, 8h, e) $POCl_3$, DMAP, 80 - 90 ^{0}C. f) H_2O, HCl, m-ethynylaniline, 40 ^{0}C.

The *O*-alkylation of esters **2** with 1-bromo-2-methoxyethane in DMF and K_2CO_3as catalysts at 100 ^{0}C afforded **3** with high yield. Adding a phase transfer catalyst as TBAC (tetrabutyl ammonium chloride) does not help to improve the yield of the reaction. Nitration of compound **3** in nitric acid and glacial acetic acid give only derivatives **4** in 90.7 % yield. The structure of **4** was determined by ^{1}H-NMR and compared to the reported data [7]. Reduction of nitro groups was carried out at room temperature with catalytic Pd/C10 % and ammonium formate, gave amine **5** with high yield (95 %).

The synthetic pathway from **2-5** can be done directly on the crude products without purification by silica gel column, while avoiding the use of flammable and explosive hydrogen gas. Cyclization occured in formamide in the presence of ammonium formate (**6**, 79.6 %). ^{1}H and ^{13}C NMR spectra of **6** confirmed the quinazolone structure with one more insaturated CH group (1 singlet at δ_H 8.04 ppm,and δ_C 148.9 ppm) and a shifting to higher field of carbon C = O signal, compared with the spectra of **5**. The molecular ion peaks from ESI-MS spectrum of **6** was matched with the calculated mass ($[M+H]^+$*m/z* 295,11, calculated 295.13) and corresponding to the loss of 1 and 2 methoxyethyl group (*m/z* 237.07 and 178.99).

For the preparation of 4-chloro-derivatives quinazoline 7, oxalylchloride and $POCl_3$ were used as clorination reagent. When using $POCl_3$, higher reaction yield was obtained comparing to the reaction using oxalylchloride even in large excess of reagent. In the final step, erlotinib was prepared by the substitution of ethynyl aniline in acidic medium, so the product is obtained under hydrochloride salt form as white crystals (**1**, 87.5 %). The overall yield of erlotinib hydrochloride preparation was 51 % with 6 steps. Chemical structure of compound **1** was determined by NMR (1, 2D) and MS spectra. NMR spectra of **1** showed the presence of the quinazoline ring signals together with aromatic ring signals owning 3 protons and acetylyl

group (1 singlet of CH at δ_H 4.18 ppm (Figure 3)2 carbon at δ_C 83.5 ppm and 80.4 ppm). (+) - ESI-MS spectrum (Figure 4) gave molecular ion peak $[M+H]^+$at *m/z* 394.34, including ion peaks corresponding to the loss of one MeO-C_2H_3 group at *m/z* 336.25 $[(M+H)-C_3H_6O]^+$, or two MeO-C_2H_3 groups at *m/z* 278.03 $[(M+H)-2C_3H_6O]^+$. HPLC analysis (Figure 5) showed that the synthetic erlotinib hydrochloride had 99.5 % purity.

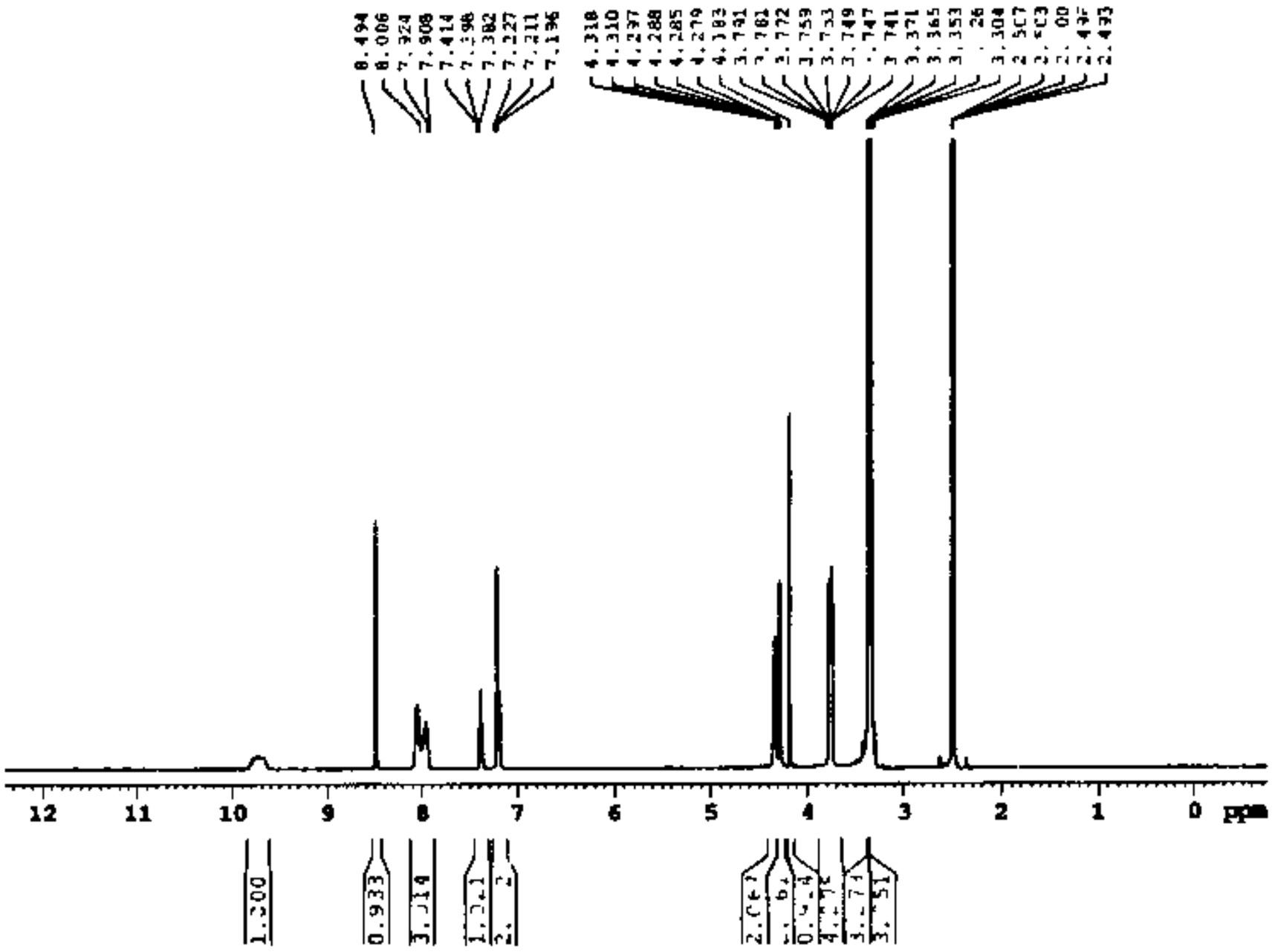


Figure 3. ^{1}H NMR spectrum of erlotinib hydrochloride (**1**) (500 MHz, DMSO d6).

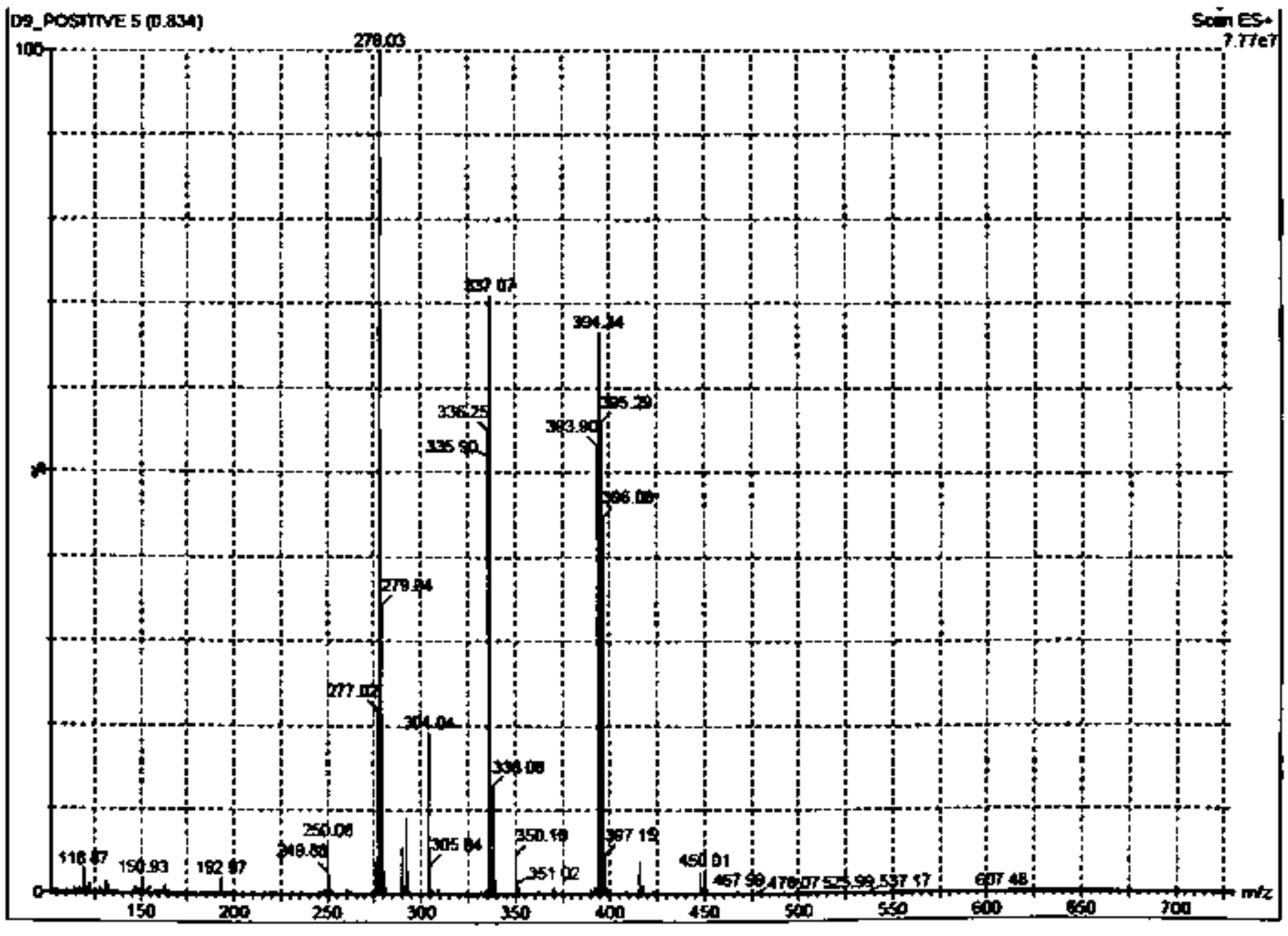
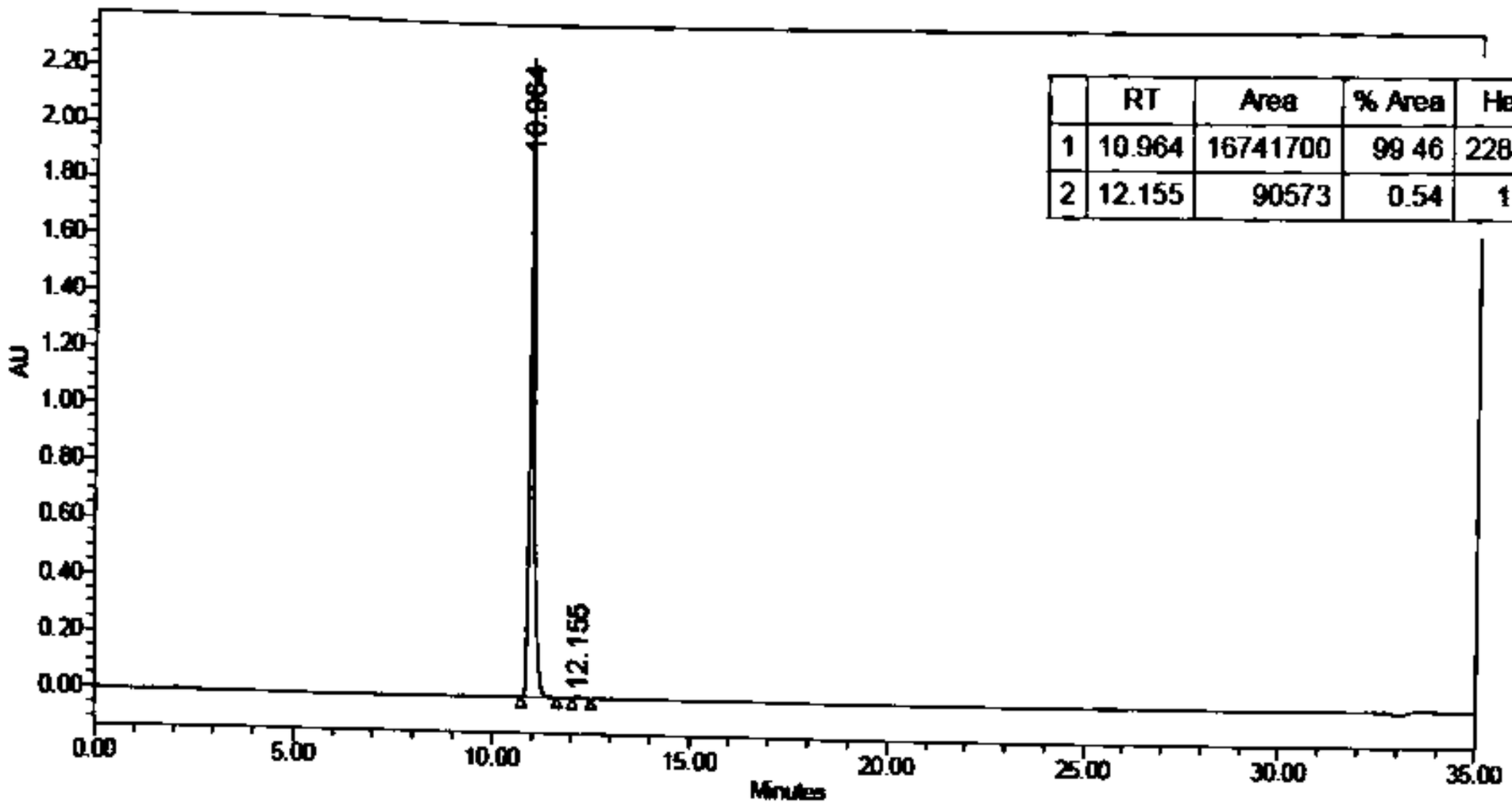


Figure 4. (+) ESI-MS spectrum của erlotinib hydrochloride (**1**).



	RT	Area	% Area	Height
1	10.964	16741700	99.46	2285992
2	12.155	90573	0.54	11479

Figure 5. HPLC chromatogramoferlotinib hydrochloride (**1**).

4. CONCLUSIONS

Erlotinib hydrochloride was prepared by an efficient and economical process in 6 stepswith overall yield of 51 %. The products from each stepcan be used as crude form without purification by silica gel chromatography for the next step.The synthetic process can be scaled up for the trial production.

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TÓM TẮT

NGHIÊN CỨU TỔNG HỢP ERLOTINIB HYDROCHLORIDE

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Erlotinib hydrochloride (Tarceva) là một thuốc điều trị trúng đích được dùng cho điều trị ung thư phổi dạng tế bào không nhỏ, ung thư tụy và một số loại ung thư khác. Trong bài báo này, erlotinib hydrochloride đã được nghiên cứu tổng hợp theo một phương pháp đơn giản, hiệu quả bao gồm 6 giai đoạn phản ứng, với hiệu suất tổng thể là 51 %.

Từ khóa: Erlotinib, ung thư phổi, phương pháp tổng hợp.