An Improved Procedure for the Preparation of Poziotinib

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Abstract—1. Objective: To improve the synthetic procedure of the anticancer drug poziotinib. 2. Methods: The poziotinib was synthesized in six stages starting from 7-methoxy-4-oxo-3,4-dihydroquinazolin-yl acetate (2) by chlorinated, ammonification, hydrolysis, condensation, take off Boc and amidation reaction. Compound 2 reacted with POCl3 followed by ammonification to afford 8. Compound 8 reacted with ammonia water in methanol to obtain 9 which passes through the condensation reaction to obtain 6. Compound 6 took off Boc in the CF3COOH followed by amidation reaction to afford the poziotinib. Results: The anticancer drug poziotinib was synthesized by using 7-methoxy-4-oxo-3,4-dihydroquinazolin-yl acetate as starting material in 6 steps. The structures were characterized by 1H NMR and ESI-MS. 4. Conclusion: The starting material is very easy to get, the reaction conditions are moderate and the operation is convenient. The reaction steps are shortened and the overall yield is 37.2 %. This method is suitable for industrial producing.

Keywords-poziotinib; anticancer activity; quinazoline; epidermal growth factor receptor; synthesis

I. INTRODUCTION

The epidermal growth factor receptor (EGFR) family of receptor tyrosine kinases has been implicated in a variety of cancers. In particular, activating mutations such as the L858R point mutation in exon 21 and the small in-frame deletions in exon 19 of the EGFR tyrosine kinase domain are correlated with sensitivity to EGFR tyrosine kinase inhibitors in non-small cell lung cancer (NSCLC) patients[1-4]. Poziotinib **1**, a highly potent, and orally bioavailable inhibitor of human epidermal growth factor receptors HER_1, HER_2, and HER_4 tyrosine kinase, was developed in a late-stage clinic trial for the prevention and treatment of patients with NSCLC including clinical limitation caused by acquired mutation (EGFR T790M), breast cancer and gastric cancer by Hanmi Science Co., Ltd[5-7]. It could be marketed for the treatment of advanced solid tumors as a new-generation anticancer.

Poziotinib combined with 5-fluorouracil, cisplatin, paclitaxel, or gemcitabine showed a synergistic inhibitory effect on the HER_2-amplified and on some of the HER_2-nonamplified breast cancer cells[8-10]. Poziotinib could be a promising treatment for HER_2-amplified breast cancer as a single agent or in combination with cytotoxic agents and can be a candidate for treatment of HER_2-nonamplified breast cancer in combination with cytotoxic agents[11-12].

Additionally, poziotinib shows an excellent efficacy in a variety of EGFR- and HER_2-dependent tumor xenograft models, including erlotinib-sensitive HCC827 NSCLC cells, erlotinib-resistant NCI-H1975 NSCLC cells, HER_2 overexpressing Calu-3 NSCLC cells, NCI-N87 gastric cancer cells, SK-Ov3 ovarian cancer cells and EGFR-overexpressing A431 epidermoid carcinoma cancer cells[13-17]. On the basis of these preclinical results, poziotinib is the most potent pan-HER inhibitor, which will be advantageous for the treatment of patients with NSCLC including clinical limitation caused by acquired mutation (EGFR T790M), breast cancer and gastric cancer[18-22].

In2008, BANG et al. reported a strategy for the synthesis of **1** (Fig .1)[23]. In the process of the synthesis of intermediates **4**, **3** reacted with NH_3/MeOH to afford chlorinated products. In the Fig .1, the compound **6**, as a key intermediate, was prepared from **4** with Ph_3P and DEAD reagent to obtain compound **5**, which is difficult to be purified in the process of separation, and it is need to use chromatographic column. Then, compound **5** reacted with 3,4-dichloro-2-fluoroaniline to affort **6**. This related method has severe drawbacks, limiting the large-scale synthesis of **1**: (a) the use of expensive NH_3/MeOH, (b) chromatographic column separation. Accordingly, this synthetic method cannot meet the demand of a large-scale preparation of **1** in terms of cost and hazard generation.

As part of the process of bringing a new active pharmaceutical ingredient (API) to market, it often requires use of an alternative synthetic strategy to the initial medicinal chemistry approach. Herein, we report a dramatically improved method for the preparation of poziotinib from the cheap 7-methoxy-4-oxo-3,4-dihydroquinazolin-yl acetate as starting material in an

overall yield of 37.2 % (Fig .2). In a certain degree, the method eliminates the use of chromatographic column separation. Therefore, the strategy has displayed its practical application potential.

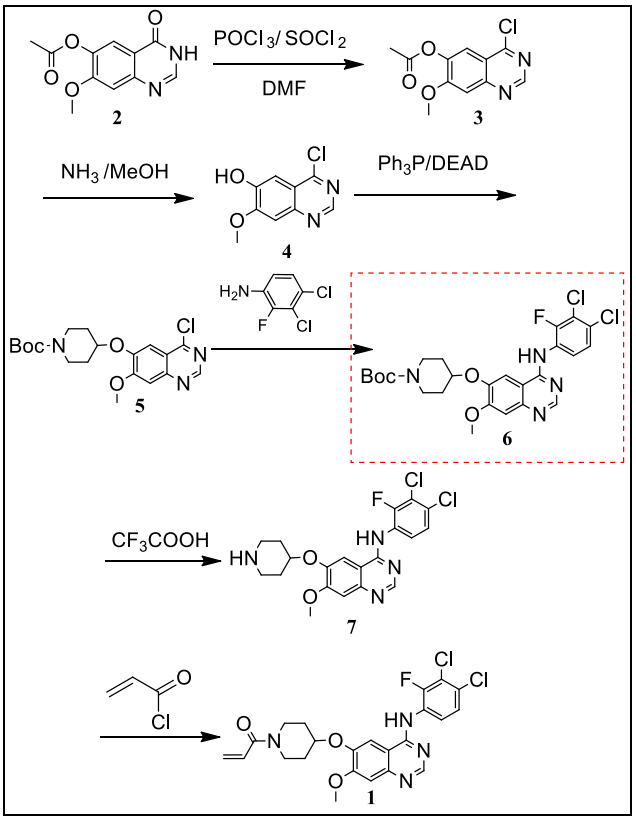

Figure 1. Reported synthetic route to poziotinib.

II. EXPERIMENTAL

^{1}H-NMR spectra were recorded on a Bruker spectrometer at 400 MHz, and the chemical shifts were reported as *d* values in parts per million relative to tetramethylsilane (TMS) as an internal standard. Mass spectra were recorded on Finnigan Trace Ms mass spectrometer. All solvents and reagents were obtained from commercial sources and used without further purification. Thin-layer chromatography(TLC) was performed on silica-gel plates (HF_{254}), and TLC visualizations were performed with ultraviolet (UV) light.

Preparation of 4-(3,4-dichloro-2-fluorophenylamino)-7- methoxyquinazolin-6-yl acetate (**8**) 7-methoxy-4-oxo-3,4-dihydroquinazolin-yl acetate (10 g, 0.04 mol) was added to toluene (250 ml) and Et_3N (15 ml). Phosphorusoxy chloride (10 ml) was added at 80 ℃, followed by stirring for 2 hours. 3,4-dichloro-2-fluoroaniline (7.0 g, 0.039mol) were added to the resulting mixture, followed by stirring for 3 hours (TLC monitor). Upon completion of the reaction, the resulting mixture was cooled to 25 ℃. The solid thus obtained was filtered under a reduced pressure. Isopropanol (180 ml) was added to the solid, which was then stirred for 2 hours(TLC monitor). The resulting solid was filtered. The solid was dried at 50 ℃ in an oven to produce the compound of **8** (16 g, yield: 94.6%) as a brown powder. ^{1}H-NMR (DMSO-d_6, 400 MHz, ppm) δ 8.91 (s, 1H), 8.73 (s, 1H), 7.55- 7.68 (m, 3H), 4.03 (s, 3H), 2.35 (s, 3H); ESI-MS m/z: 396 $[M + H]^+$.

Preparation of 4-(3,4-dichloro-2-fluorophenylamino)-7- methoxyquinazolin-6-ol (**9**)4-(3,4-dichloro-2-fluorophenylamino)-7-methoxyquinazolin-6-yl acetate (16 g, 0.04 mol) was admixed with methanol (500 ml). The mixture was cooled to 10 to 15 ℃, added with an ammonia solution (90 ml), and stirred for 1 hours at 25 ℃. The solid thus obtained was filtered and washed with a mixed solvent of methanol (30 ml) and water (30 ml). The resulting solid was dried at 50 ℃ in an oven to produce **9** (12.6 g, yield: 88.1%) as a white powder. ^{1}H-NMR (DMSO-d_6, 400 MHz, ppm) δ 9.53 (br, 2H), 8.38 (s, 1H), 7.62 (s, 1H), 7.50-7.63 (m, 2H), 7.19 (s, 1H), 4.01 (s, 3H); ESI-MS m/z: 354 $[M + H]^+$.

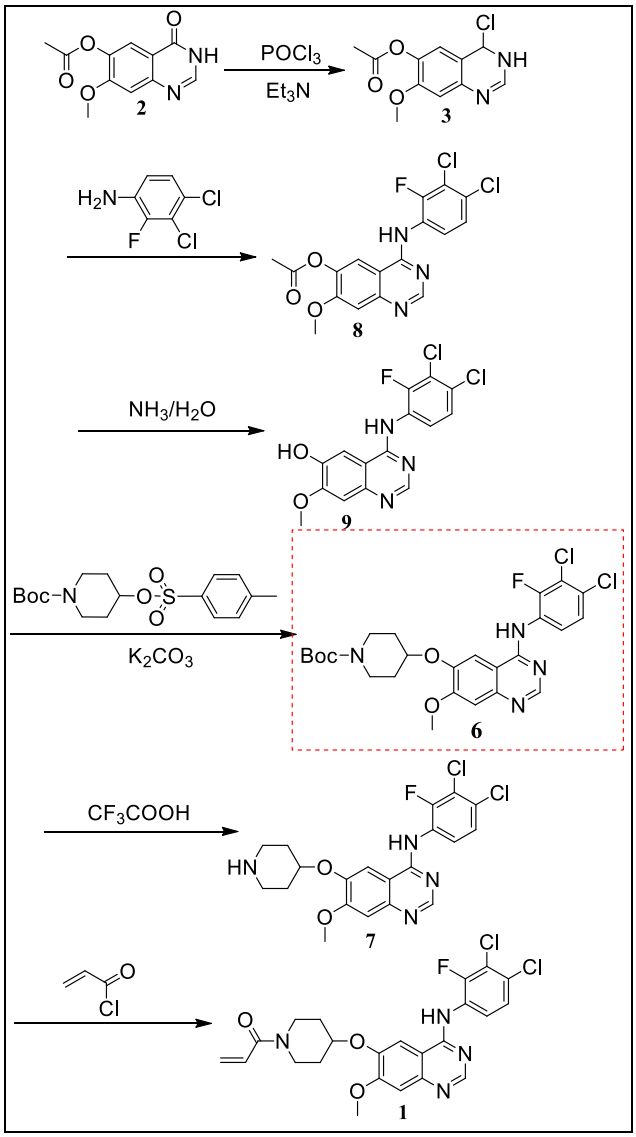

Figure 2. Alternate route to the synthesis of poziotinib.

Preparation of tert-butyl l-4-(4-(3,4-dichloro-2-fluorophenylamino)-7-methoxyquinazolin-6-yloxy) piperidin-l-carboxylate (**6**) 4-(3,4-dichloro-2-fluorophenylamino)-7-methoxyquinazolin-6-ol (12.6 g, 0.036mol) was admixed with N,N-dimethylformamide (80 ml) under stirring, followed by addition of tert-butyl 4-(tosyloxy)piperidin-l-carboxylate (15 g, 0.042 mol) and

potassium carbonate (15 g, 0.113) to the mixture. The reaction temperature was raised to 90 ℃, and the mixture was stirred for 3 hours(TLC monitor). The temperature of the resulting solution was cooled to 25 ℃, and water (160 ml) was slowly added thereto. The solid thus obtained was filtered and dried. The solid was dissolved in a mixed solvent (220 ml) of dichloromethane and methanol, followed by stirring for 2 h. The resulting mixture was filtered through a Celite pad, distilled under a reduced pressure, added with acetone (75 ml), and stirred for 6 hours. The resulting solid was filtered and washed with acetone (20 ml). The solid was dried at 40 ℃ in an oven to produce **6** (14.2 g, yield: 74.3%) as a yellowish-brown powder. ^{1}H-NMR (DMSO-d_6, 400 MHz, ppm) δ 8.69 (s, 1H), 8.47 (t, 1H), 7.34- 7.29 (m, 2H), 7.20 (s, 1H), 4.63-4.60 (m, 1H), 3.82 (s, 3H), 3.83-3.76 (m, 2H), 3.37-3.29 (m, 2H), 1.99-1.96 (m, 2H), 1.90-1.84 (m, 2H), 1.48 (s, 9H) ; ESI-MS m/z: 537 $[M + H]^+$.

TABLE I. PERCENT OF **3** CONVERSION IN DIFFERENT CHLORINATED REAGENTS

Entry	Percent of 3 Conversion in Different Chlorinated Reagents			
	Chlorinated reagents	*Temp(℃)*	*Time(h)*	*Conversion(%)*
1	$SOCl_2$/ Et_3N	90	8	78
2	PCl_3/ Et_3N	110	10	15
3	$POCl_3$/ Et_3N	80	2	95
4	$SOCl_2$	90	8	56
5	$POCl_3$	80	2	82

Preparation of N-(3,4-dichloro-2-fluorophenyl)-7-methoxy-6-(piperidin-4-yloxy)quinazolin-4-amine (**7**) Acetone (50 ml) was added to tert-butyl 4-(4-(3,4-dichloro-2- fluorophenylamino)-7-methoxyquinazolin-6-yloxy)piperidin-l-carboxylate (14.2 g, 0.026 mol), which was then stirred. The mixture was added with CF_3COOH (30 ml) for 20 minutes and stirred for 8 hours(TLC monitor). Upon completion of the reaction, the resulting mixture was concentrated, and the resulting solid thus obtained was washed with acetone (20 ml). The solid was dried at 50 ℃ in an oven to produce **7** (11 g, yield: 95.2 %) as a yellowish powder. ^{1}H-NMR (DMSO-d_6, 400 MHz, ppm) δ 12.88 (bs, 1H), 9.39 (bs, 1H), 9.21 (bs, 1H), 9.05 (s, 1H), 8.89 (s, 1H), 7.55-7.72 (m, 2H), 7.38 (s, 1H), 5.05- 5.12 (m, 1H), 4.02 (s, 3H), 3.19-3.30 (m, 4H), 2.28-2.31 (m, 2H), 1.90-1.97 (m, 2H) ; ESI-MS m/z: 437 $[M + H]^+$.

Preparation of l-(4-(4-(3,4-dichloro-2fluorophenyl-amino)-7-methoxyquinazolin-6-yloxy)piperidin-l-yl)prop-2-en-1-one (**poziotinib , 1**)N-(3,4-dichloro-2-fluorophenyl) -7-methoxy-6-(piperidin-4-yloxy)quinazolin-4-amine dihydrochloride (11 g, 0.025mol) and sodium hydrogen carbonate (8 g, 0.095mol) were added to a mixed solvent of tetrahydrofuran (90 ml) and water (120 ml), and the temperature of the reaction mixture was cooled to 0 ℃ with iced water. Acryloyol chloride (3 ml, 0.037 mol) diluted with tetrahydrofuran (50 ml) was slowly added to the reaction mixture over 50 minutes, followed by stirring at 0 ℃ for 2 h (TLC monitor). Upon completion of the reaction, aqueous acetone (200 ml) was added to the resulting mixture, which was stirred for 24 hours and filtered to produce **1** as a yellowish-brown powder. The solid thus obtained was dissolved in a mixed solvent of dichloromethane (20 ml) and methanol (10 ml), added with ethyl acetate (120 ml), and stirred for 24 hours. The resulting solid was filtered and washed with ethyl acetate (10 ml). The solid was dried at 50 ℃ in an oven to produce **1**(7.8 g, yield: 63.1%) as a white powder. ^{1}H-NMR (DMSO-d_6, 400 MHz, ppm) δ 8.66(s, 1H), 8.37(t, 3H), 7.29(m, 3H), 6.63(m, 1H), 6.25(m, 1H), 5.70(m, 1H), 4.72(m, 1H), 4.01(s, 3H), 3.86(m, 2H), 3.57(m, 2H), 1.88(m, 4H) ; ESI-MS m/z: 491 $[M + H]^+$.

III. RESULTS AND DISCUSSION

In the medicinal chemistry approach, the key intermediate **6** was synthesized in four stages starting from 7-methoxy-4-oxo-3,4-dihydroquinazolin-yl acetate **2** as shown in Fig .2. In the first step, chlorinated product **3** was generated by the addition of chlorinated reagents to a solution of **2** in toluene followed by addition of 3,4-dichloro-2-fluoroaniline to afford **8**. Compound **8** was reacted with alkali liquor in methanol at 25 ℃ for 1 h. NH_3/H_2O was found to be good in terms of alkalic system. In the next stage, **9** was treated with tert-butyl 4-(tosyloxy)piperidin-l-carboxylate in DMF, and then the reaction mixture was added to H_2O to afford **6**.

In the synthesis of **3**, we investigated the reaction in different chlorinated reagents viz. $SOCl_2$, PCl_3, and $POCl_3$ (Table I). As seen in Table I, reaction was relatively slow, and conversion to product was not satisfactory in either $SOCl_2$, or PCl_3 chlorinated reagents. Reaction in $POCl_3$ was very good. In the case of $POCl_3$, the reaction mixture was acidic; it was necessary to add Et_3N to afford **3**.

IV. CONCLUSION

It is worth noting that we had carried out significant developmental work on the medchem approach with minimal changes to the route of synthesis. These key changes in the process made significant impact on yield, time, and environmental aspects. In summary, we have developed an efficient process for largescale manufacturing of poziotinib through a convenient approach.

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