

A method with safety and convenience to synthesize Regorafenib

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Abstract. To date, three methods have been developed to synthesize Regorafenib, a drug which can effectively cure colorectal cancer. However, all of them have drawbacks: some are complicated to obtain due to the high requirements the drugs need. And some even contain dangerous processes or poisonous drugs that are difficult to store. Also, there is a way that contains a lot of by-products, which makes extracting Regorafenib very difficult. In this essay, one of the synthetic routes and the data from the experiment will be used to test whether it works. Furthermore, the selected route was improved. Here, the best base on the reaction is t-BuOK; 1.1 eq of potassium tert-butoxide was preferred; the most appropriate temperature is 100°C; the ZA1 and SM3 feeding ratio is preferably 1.10 eq; the reaction time can be chosen 3-7 h.

Keywords: Regorafenib, nucleophilic, substitution, electronegative, recrystallize.

1. Introduction

Targeted therapy is becoming more significant in the current hepatocellular carcinoma therapeutic paradigm. However, liver cancer is frequently discovered in the middle or advanced stages and has a high incidence rate worldwide [1]. The value of targeted therapy is increasing. Sorafenib, a molecularly targeted medication, has recently been successful in treating liver cancer [2]. Nevertheless, the rate of illness control remains low, and it is clear that drug resistance and adverse effects exist.

Each year, more than 600,000 people worldwide are diagnosed with colorectal cancer, and almost 1.25 million people pass away from it. At least 50% of patients experience metastases, and most tumors cannot be removed [3]. Chemotherapy based on fluoropyrimidines, oxaliplatin, and irinotecan (given in combination and sequentially), as well as monoclonal antibodies that target vascular endothelial growth factor (VEGF; bevacizumab), are the standard treatments for these individuals [4]. Cetuximab and panitumumab, two monoclonal antibodies that target the EGFR, are also used in patients with KRAS wild-type tumors [5,6]. Because many individuals maintain acceptable performance status and may be candidates for additional therapy, more choices are required for patients with disease progression despite using all currently available standard medicines [7].

Numerous signaling pathways, including receptor tyrosine kinases (such as EGFR, VEGF receptor, platelet-derived growth factor receptor (PDGFR), and fibroblast growth factor receptor (FGFR)), as well as downstream signaling cascades, have been linked to the onset and progression of colorectal cancer [6]. Regorafenib is a brand-new oral multichines inhibitor that inhibits the activity of a number of protein kinases, such as those that control tumor angiogenesis: VEGFR1, also known as FLT1, oncogenesis KIT, RET, RAF1, BRAF, and BRAFV600E, and the microenvironment in tumors (PDGFR and FGFR). Regorafenib has demonstrated antitumor activity in pre-clinical investigations, notably in colorectal cancer model organisms [7,8].

This article will focus on one method for Regorafenib preparation. Then, how to make the reaction more effective was inferred. The effect of bases on the reaction, the amount of potassium tert-butoxide, the ZA1 and SM3 feeding ratios, reaction time, and the reaction temperature were optimized. Here, this will not only enhance the preparation of Regorafenib, but may also provide guidance for synthesizing other similar drugs.

2. Mechanism

The description of the mechanism which was behaved in this paper are as follows:

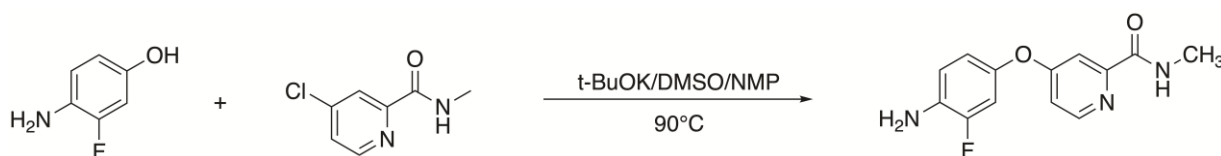


Figure 1. The first step of the synthesis pathway.

The synthesis pathway can be divided into three steps. The first step is the nucleophilic substitution reaction of 4-amino-3-fluorophenol (C_6H_7FNO) and n-methyl-4-chloro-2-pyridinecarboxamide ($C_7H_7ClN_2O$) to form 4-(4-amino-3-fluorophenoxy)-N-methylpyridine-2-carboxamide ($C_{13}H_{12}FN_3O_2$) (Figure 1). Because t-BuOK (potassium tert-butoxide $C_4H_{12}KO$) is a strong base, it takes away the hydrogen ion from the hydroxyl group of 4-amino-3-fluorophenol resulting in only the oxygen negative ion. In addition, in the case of n-methyl-4-chloro-2-pyridinecarboxamide, the electrons of the covalent bond with chlorine form a shift in favor of the chlorine because the chlorine element is more electronegative than the carbon atom so that the carbon attached to the chlorine shows a relatively positive charge. The oxygen negative ion then becomes a nucleophilic attack reagent to attack the carbon positive ion on n-methyl-4-chloro-2-pyridinecarboxamide to form an intermediate. Since there are 5 chemical bonds attached to the carbon, breaking the 8-electron stability, the chloride is taken off and the chloride negative ion and hydrogen ion form a hydrochloric acid which becomes a by-product.

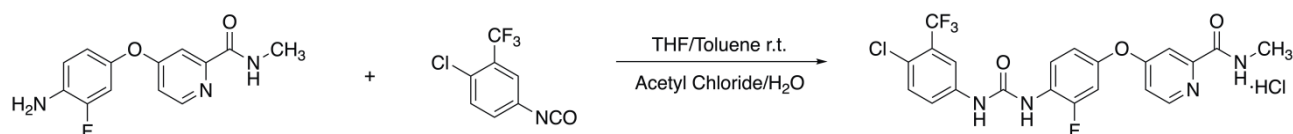


Figure 2. The second step of the synthesis pathway.

The second step is the nucleophilic addition reaction of 4-(4-amino-3-fluorophenoxy)-N-methylpyridine-2-carboxamide ($C_{13}H_{12}FN_3O_2$) and 4-chloro-3-(trifluoromethyl)isocyanate ($C_8H_3ClF_3NO$) (5) to produce the hydrochloride form of regorafenib ($C_{21}H_{15}ClF_4N_4O_3$) (Figure 2). First, because the carbon and oxygen of 4-chloro-3-(trifluoromethyl) isocyanate ($C_8H_3ClF_3NO$) form a double bond and the electronegativity of oxygen is higher than that of carbon, the electrons will be shifted toward oxygen, resulting in a partially positive carbon ion. Then, since the nitrogen of 4-(4-amino-3-fluorophenoxy)-N-methylpyridine-2-carboxamide ($C_{13}H_{12}FN_3O_2$) has a lone electron on it, the lone electron becomes a nucleophilic attacking agent to attack the partially positively charged carbon ion to form a chemical bond. Since the nitrogen breaks through the eight-electron stability, in this case, the original hydrogen is removed to reach the eight-electron stability. This also happens to carbon, which forms five bonds and breaks the eight-electron stability, so it breaks the double bond with nitrogen and becomes a single bond. The nitrogen then takes on a negative charge, and the positively charged hydrogen then forms a chemical bond with the nitrogen. The hydrochloride form of regorafenib ($C_{21}H_{15}ClF_4N_4O_3$) is formed. It is worth noting that THF (tetrahydrofuran C_4H_8O) is a nonprotic solvent of medium polarity, and its presence prevents the hydrogen coming off the nitrogen from combining with the oxygen on the carbon-oxygen double bond to become an alcohol.

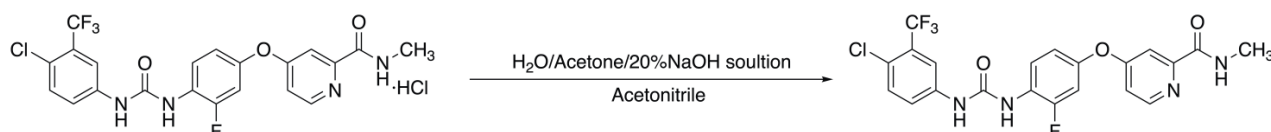


Figure 3. The third step of the synthesis pathway.

The last step is the neutralization reaction of the hydrochloride form of regorafenib ($C_{21}H_{15}ClF_4N_4O_3$) with 20% sodium hydroxide (NaOH) solution to remove the hydrochloric acid to

form the original regorafenib ($C_{21}H_{15}ClF_4N_4O_3$) (Figure 3). This process involves the addition of acetonitrile (CH_3CN) to recrystallize the regorafenib solution to improve the purity of the product.

3. Experimental section

DMSO-I (60 g) was added into the three-mouth bottle, and nitrogen was replaced three times. The temperature was raised to $60 \pm 1^\circ C$, and nitrogen was replaced 3 times. Add 6 g sm1 and 7.3 g sm2, and replace them with nitrogen three times. Heat to $95 \pm 1^\circ C$. Add 180 mL purified water to another larger three-mouth bottle and preheat to $80 \pm 2^\circ C$. In a 100 ml beaker, 12 g DMSO-II and 6 g NMP mixed solvent was added, and nitrogen was replaced three times. Then 5.3 g potassium tert-butanol was added, stirred, and then dissolved for later use; The temperature in the smaller three-mouth bottle was raised to $95 \pm 1^\circ C$, and the prefabricated potassium tert-butanol solution was slowly added in the nitrogen environment to control the internal temperature within $105^\circ C$. After adding drops, samples were taken at 2 h for inspection. After the reaction, turn off the heating, and take 150 ml preheated purified water from the larger three-mouth bottle and add it into the smaller three-mouth bottle to quench the reaction. After stirring for 10 min, transfer the reaction liquid from the smaller three-mouth bottle to the larger three-mouth bottle, and then add 12mL acetonitrile. The reaction solution was stirred at $80 \pm 1^\circ C$. Cooling crystallization for 24 h. The reaction kettle was washed with a mixed solvent of 12 ml acetonitrile and 36ml purified water using a Brucella funnel filter, and the washing solution was divided into two washing filter cakes. $70^\circ C$ and dry. Then the product was gotten and was weight 6.93 g.

4. Results and discussion

4.1. Product Inspection

After obtaining the final product, IR spectrum was applied to analyze the outcome.

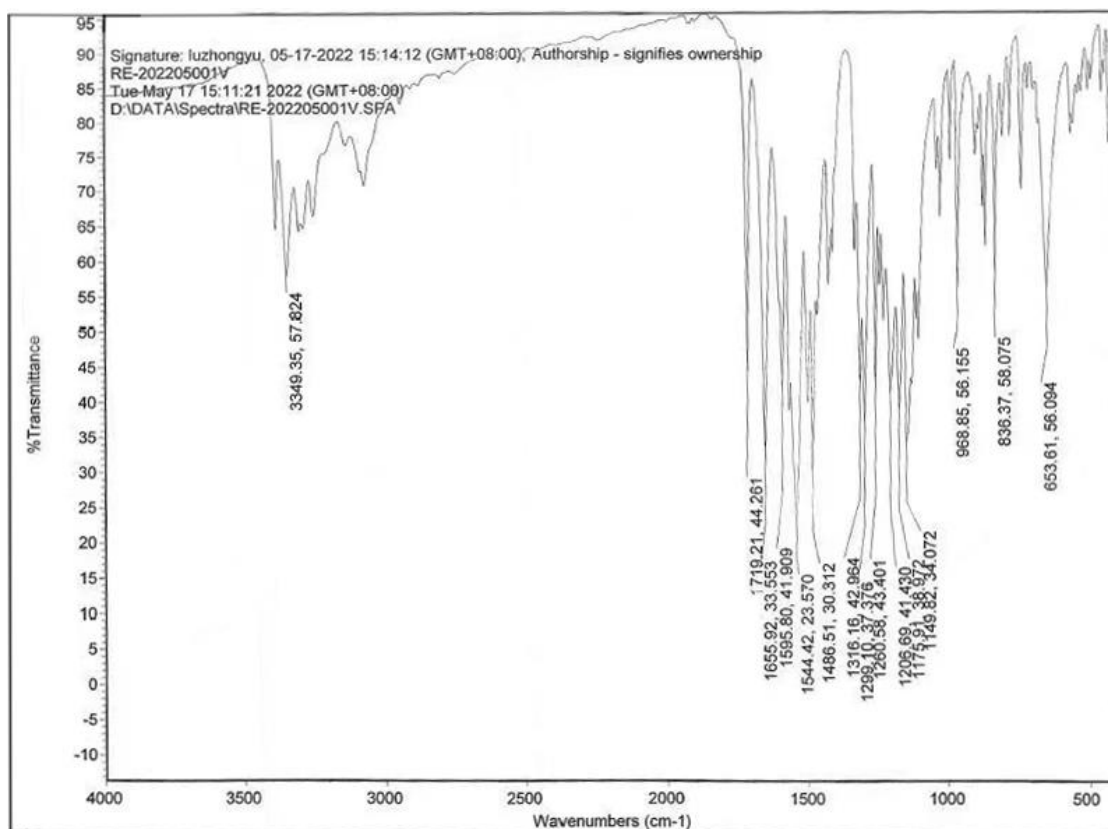


Figure 4. The IR spectrum of Regorafenib.

By comparing the spectra obtained by IR with those obtained by Regorafenib in Figure 4, we can conclude that we have successfully synthesized Regorafenib.

4.2. Screening of base species in the synthesis step

To examine the effect of the type of base on the reaction. The effects of K_2CO_3 , NaOH, and tBuOK as bases on the reactions were examined separately.

Table 1. Three Trials comparing for different bases

Trial	Base	Time of reaction	SM2	ZA1	Impurity IMP2 (%)	Impurity IMP3 (%)
1	K_2CO_3	2.0 h	86.55	11.46	1.41	N/A
2	NaOH	2.0 h	23.97	51.26	22.41	2.29
3	t-BuOK	2.0 h	2.31	96.83	0.56	0.21

In Table 1, it was found that K_2CO_3 was less basic and poorly converted, with 86.55% SM2 remaining after 2.0 h of reaction; more impurities were generated in NaOH; t-BuOK was more basic and had better solubility in NMP, so it was more convenient to feed, while the amounts of impurities generated in the reaction solution was smaller, so t-BuOK was preferred for the bases.

4.3. Screening of the amount of potassium tert-butoxide in the synthesis step

To examine the effect of the amount of potassium tert-butoxide on the reaction, The amount of potassium tert-butoxide was examined at 1.00eq, 1.05eq and 1.10eq of the SM1 molar amount for ensuring that other conditions remained unchanged were studied.

Table 2. Three trails comparing for different Potassium tert-butoxide feeding ratio

Trial	Potassium tert-butoxide feeding ratio	SM2 residual peak area(%)	ZA1 product peak area(%)	Impurity IMP2(%)	Impurity IMP3 (%)
1	1.00eq	2.311	96.83	0.56	0.21
2	1.1eq	0.537	98.16	0.69	0.49
3	1.2eq	0.037	96.27	0.83	2.57

In Table 2, it was found that as the amount of alkali gradually increased, the remaining amount of SM2 gradually decreased, and when the amount of alkali increased to 1.1 eq, the highest ZA1 content in the reaction solution, and then continuing to increase the amount of alkali equivalent would cause a significant increase in impurity IMP3, and from the results and cost considerations, 1.1 eq was preferred.

4.4. Choice of reaction temperature in the synthesis step

To examine the effect of different reaction temperatures on the reaction, three temperatures, $80 \pm 2^\circ C$, $90 \pm 2^\circ C$, and $100 \pm 2^\circ C$ were evaluated.

Table 3. Three trials comparing for different temperature

Trial	Temperature	Time of reaction	SM2 residual peak area(%)	ZA1 product peak area(%)	Impurity IMP2(%)
1	$80 \pm 2^\circ C$	2.0 h	6.358	92.82	0.56
2	$90 \pm 2^\circ C$	2.0 h	6.373	93.06	0.32
3	$100 \pm 2^\circ C$	2.0 h	2.766	96.32	0.20

As the reaction temperature increases, the remaining amount of SM2 decreases and the content of ZA1 increases, while the content of impurity IMP2 decreases (Table 3). Therefore, the reaction temperature is preferably $100^\circ C$.

4.5. Examination of ZA1 and SM3 feeding ratios in the synthesis step

As: too much ZA1 residue may cause an increase in impurity content, while if too much SM3 is added, the cost is too high, so the ratio of the two needs to be controlled. Therefore, the ratios of ZA1 and SM3, 1:1, 1:1.05, and 1:1.1, were further evaluated.

Table 4. Three trials comparing for different ZA1/ SM3 molar ratio

Trial	ZA1/ SM3 molar ratio	ZA2 product peak area(%)	ZA1 residual peak area(%)	SM3 residual peak area(%)	Impurity IMP3 (%)
1	1.00:1.00	96.40	1.14	0.26	0.29
2	1.00:1.05	96.04	0.10	1.46	0.51
3	1.00:1.10	95.16	0.02	2.59	0.65

From the reaction solution data in Table 4, it can be seen that the residual amount of ZA1 gradually decreases with the increase of SM3, while the residual amount of SM3 also gradually increases. Based on the consideration of mainly controlling the residual amount of Z1, the feeding molar ratio of SM3 and ZA1 is preferably 1.10eq.

4.6. Examination of the reaction time in the synthesis step

What is more, effect of the product and impurity content with increasing reaction time was examined. In this experiment, other conditions were kept constant, and samples were taken at different reaction times to detect the residues of raw materials and the formation of impurities.

Table 5. Three trials comparing for different time of reaction

Trial	Time of reaction	ZA1 residual peak area	Impurity IMP3	Impurity IMP4	ZA2 product peak area
1	1.0 h	0.51%	0.77%	0.40%	95.83%
2	3.0 h	0.26%	1.09%	0.40%	97.07%
3	5.0 h	0.24%	1.12%	0.40%	97.23%
4	7.0 h	0.24%	1.11%	0.41%	97.19%

It can be seen in Table 5 that with the prolongation of the reaction time, the product purity increased and the remaining amount of ZA1 decreased gradually from 1h to 3 h, but there was no significant increase in the stage of 3-7 h. Therefore, the reaction time can be chosen from 3-7 h.

5. Conclusion

In this study, one synthesis process of regorafenib was discussed in terms of routes and was investigated. A preliminary optimization study of the synthesis conditions was carried out to investigate the effects of various reaction conditions on the reaction results and impurities: the optimum reaction base is t-BuOK, 1.1 eq of potassium tert-butoxide was favored, 100 °C was the ideal temperature, 1.10 eq of ZA1 and SM3 was liked as the feeding ratio, and 3–7 hours can be selected for the reaction period. Finally, the preparation of regorafenib was carried out. The method in this article will enhance the efficiency and stability of regorafenib synthesis and will guide the synthesis of other similar drugs.

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