Microwave Reaction Improved Heterocyclization of Quinazolinone Ring in Synthesis of Erlotinib Analogues

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Abstract:

**Background** Tinibs were a kind of important epidermal growth factor receptor (EGFR) inhibitors used as potential therapeutic agents in treating non-small cell lung cancer (NSCLC) in clinic. The drug resistance of clinical used tinibs made the development of more active tinib analogues an attractive field in research. Quinazoline ring was regarded as the key fragment in tinibs and quinazolinone was indispensable intermediate in the synthesis of quinazoline. Thus, synthesis of quinazolinone intermediates was a key step which would further limit the overall yield of final product of tinib analogues. However, the commonly used synthetic
scheme was somewhat complicated and time consuming with relatively low yield in heterocyclization of quinazolinone and its derivatives.

**Aim** In this work, we intended to explore an effective way to improve synthesis of heterocyclization of 6,7-bis(2-methoxyethoxy)quinazolin-4(3H)-one (compound 5), the key fragment of erlotinib analogues, in both reaction procedure and yield, thus to provide reference to synthesis of other quinazolinone derivatives.

**Methods** A simple microwave-assisted one-pot reaction was employed to improve the synthesis of heterocyclization of quinazolinone ring. The reaction conditions, including microwave power, temperature and time of reaction, were screened to achieve high yield under simple operation.

**Results** 6,7-bis(2-methoxyethoxy)quinazolin-4(3H)-one (compound 5) was successfully synthesized from starting material of 4,5-bis(2-methoxyethoxy)-2-nitrobenzonitrile by microwave reaction, which was finished in 1 hour just by one step. The yield of heterocyclization was increased from 29.8% of commonly used three-step scheme to 50% herein.

**Conclusion** Microwave reaction efficiently improved synthesis of heterocyclization of 6,7-bis(2-methoxyethoxy)quinazolin-4(3H)-one into "quinazolinone in both synthetic procedure and yield. The results may provide valuable reference to synthesis of other quinazolinone derivatives.

**Key words** Quinazolinone; heterocyclization; microwave reaction.

**INTRODUCTION** At present, a variety of EGFR inhibitors of tinibs were effectively used therapy of NSCLC, such as gefitinib\(^1\), erlotinib\(^2\), icotinib\(^3\), afatinib, canertinib, vandetanib, dacomitinib and so on. However, acquired point mutations in EGFR gene, especially for T790M mutation, were commonly observed in many NSCLC cases\(^4\), which led to drug resistance and weakened therapeutic efficacy of tinibs\(^5,6\). Therefore, development of novel tinib analogues of better activity was an attractive point.

![Figure 1](image_url) Chemical structure of some effective EGFR inhibitors of tinibs.

The tinib drugs were reported to achieve antitumor effect by the quinazoline core ring binding to a hydrophobic pocket at the N-terminus of EGFR binding region\(^7,9\), indicating quinazoline to be the chief scaffold (Fig. 1). In most of the synthetic routes of these tinibs, the formation of quinazolinone intermediate was an indispensable part which thus was a key step related to the overall yield of final product. Commonly, to synthesize quinazolinone, substituted benzonitrile was used as a raw material, the reaction required at least two steps, and the reaction yield was about 8% to 30%\(^10,11\). Some researchers put efforts to enhance the yield in the synthesis of quinazolinone intermediate. Kundu et al.\(^12\), for example, got the quinazolinone intermediates by one-pot reductive cyclization in preparing antitumor quinazoline precursors, while Saari et al.\(^13\) efficiently converted 2-aminobenzonitrile into quinazolin-4(3H)-one under microwave irradiation within a short time.

In order to develop new tinib analogues of greater efficacy, we selected 6,7-bis(2-methoxyethoxy)quinazolin-4(3H)-one part of erlotinib as basic structure for modification. The two 2-methoxyethoxy chains were somewhat bulky substituent groups which might affect the reaction efficiency of heterocyclization of quinazolinone ring. By using the commonly used three-step reaction, the heterocyclization yield was 29.8%, which further affected the overall
To expect higher yield and optimal reaction route and condition, in this work, we tried one-pot microwave reaction and screened the reaction conditions to figure out its possible advantage in cyclization of quinazolinones.

MATERIALS AND METHODS
Hydroxylamine hydrochloride, indium trichloride, formamide, sodium formate dehydrate, dichloromethane (DCM), petroleum ether (PE), ethyl acetate (EA), methanol, tetrahydrofuran (THF), acetonitrile, formic acid, trimethylamine (TEA), glacial acetic acid, chloroform, nitric acid, potassium carbonate and p-Toluenesulfonyl chloride (TsCl) were reagent grade and commercially available.

Thin-layer chromatography plates of silica gel 254F were purchased from Merck. CDCl3 or DMSO-d6 was used to dissolve chemicals for 1H-NMR spectra determination at room temperature on a Bruker AV-300 spectrometer, and the spectra were reported in chemical shifts δ (ppm) relative to tetramethylsilane (δ 0.0 ppm). Positive-ion electrospray ionization (ESI) mass spectra were recorded on a Finnigan LCQ Advantage MAX mass spectrometer (4000 Q TRAP) of Applied Biosystems.

EXPERIMENTAL
In this work, 6,7-bis(2-methoxyethoxy)quinazolin-4(3H)-one of erlotinib (compound 5) was synthesized according to Scheme 1. Taking ethylene glycol monomethyl ether as starting material, compound 1 was prepared by nucleophilic substitution with TsCl in THF. In case of nitrogen protection, 3,4-dihydroxybenzaldehyde was reacted with 2-methoxyethyl-4-methylbenzenesulfonate in acetonitrile to generate 3,4-bis(2-methoxyethoxy)benzaldehyde, compound 2. The aldehyde group of compound 2 was reduced by NH2OH•HCl to yield 3,4-bis(2-methoxyethoxy)benzonitrile (compound 3) followed by a nitration reaction to obtain 4,5-bis(2-methoxyethoxy)-2-nitrobenzonitrile, compound 4. Finally, InCl3 was used as a catalyst to enable compound 4 cyclized in formamide to yield compound 5 by microwave-assisted one-pot reaction.

Scheme 1. Reagents and conditions: (I) TsCl, THF, NaOH, H2O, ice-bath, 6 h; (II) acetonitrile, K2CO3, N2 protection, 84 °C, 36 h; (III) NH2OH•HCl, HCOONa, HCOOH, 85 °C, 5 h; (IV) 65% HNO3, CH3COOH, ice-bath/4 h, 50 °C/4 h; (V) HCONH2, InCl3, MW/400W, 110 °C, 1 h.

2-methoxyethyl-4-methylbenzenesulfonate (1)
Ethylene glycol monomethyl ether 2.28 g, 30 mmol) and Sodium hydroxide (1.44 g, 36 mmol) were added into a mixture of THF and water, and treated with ice bath. After 2 hours, TsCl (5.99 g, 31.5 mmol) dissolved in THF was added dropwise to the above system under ice bath. After a reaction of 4 h, THF was removed and the residue was extracted with DCM after washed with brine. After dried over anhydrous Na2SO4, the solvent of organic layer was evaporated under reduced pressure, and the residue was separated on silica gel column by a mixture of PE:EA (9:1, v/v). After solvent evaporation, oily compound 1 was yield. (3.24 g, 47 ESI-MS m/z: 231.0 [M+H]+, 248.3 [M+NH4]+. 1H NMR (300 MHz, DMSO-d6): δ 7.79 (d, J = 8.3 Hz, 2H), 7.48 (d, J = 8.0 Hz, 2H), 4.16-4.08 (m, 2H), 3.55-3.44 (m, 2H), 3.18 (s, 3H), 2.41 (s, 3H). 13C NMR (75 MHz, DMSO-d6): δ 145.37, 132.85, 130.57, 128.06, 70.17, 69.71, 58.37, 21.50.
3,4-bis(2-methoxyethoxy)benzaldehyde (2)
To a solution of 3,4-dihydroxybenzaldehyde (6.9 g, 50 mmol) in acetonitrile, compound 1 (23.1 g, 100 mmol) and K₂CO₃ (13.8 g, 100 mmol) was added followed by evacuating air, and then the mixture was heated to 84 °C and reacted for 36 h under N₂ protection. The reaction mixture was filtered and then acetonitrile in filtrate was removed. After brine washing, EA extraction, anhydrous Na₂SO₄ drying, and concentration in turn, the residue was separated on silica gel column by a mixture of PE:EA (4:1, v/v). The eluent was vacuum evaporated to obtain compound 2 as orange oil (20.4 g, 80.3%). ESI-MS m/z: 255.3 [M+H]⁺, 277.3 [M+Na]⁺. ¹H NMR (300 MHz, CDCl₃): δ 7.97 (s, 1H), 7.40 (dt, J = 8.2, 2.6, 1.8 Hz, 2H), 6.96 (d, J = 8.0 Hz, 1H), 4.23-4.13 (m, 4H), 3.76 (dt, J = 6.2, 3.8 Hz, 4H), 3.41 (s, 6H). ¹³C NMR (75 MHz, CDCl₃): δ 190.90, 154.35, 149.16, 130.20, 126.75, 112.43, 111.70, 70.76, 70.66, 68.57, 68.55, 59.29, 59.25.

3,4-bis(2-methoxyethoxy)benzonitrile (3)
Sodium formate (2.68 g, 39.9 mmol) in formic acid (1.63 g, 35.4 mmol) was added with compound 2 (5.0 g, 19.69 mmol), then n-hexane, the mixture was reacted 4 h at 0 °C followed by another 4 h at 50 °C. Then ice water and precipitate generated. After filtration, the crude product was further recrystallized using EA to yield white solid compound 3 (3.7 g, 75.5%). ESI-MS m/z: 352.3 [M+H]⁺. ¹H NMR (300 MHz, CDCl₃): δ 7.27 (dd, J = 8.4, 2.0 Hz, 1H), 7.15 (d, J = 1.9 Hz, 1H), 6.93 (dd, J = 8.4, 2.7 Hz, 1H), 4.25-4.13 (m, 4H), 3.79 (dt, J = 4.3, 3.1 Hz, 4H), 3.45 (d, J = 0.9 Hz, 6H). ¹³C NMR (75 MHz, CDCl₃): δ 152.93, 148.90, 126.83, 119.16, 117.05, 113.51, 104.17, 70.80, 70.69, 69.05, 68.62, 59.29, 59.25.

4,5-bis(2-methoxyethoxy)-2-nitrobenzonitrile (4)
The solution of compound 3 (3.7 g, 14.8 mmol) in glacial acetic acid was added dropwise into HNO₃ (65%) pre-cooled at 0 °C, and the mixture was reacted 4 h at 0 °C followed by another 4 h at 50 °C. Then ice water and precipitate generated. After filtration and washing with ice water and then n-hexane, the filter cake was dried and compound 4 of yellow solid was yielded (2.2 g, 50%). ESI-MS m/z: 297.3 [M+H]⁺. ¹H NMR (300 MHz, CDCl₃): δ 7.87 (s, 1H), 7.29 (s, 1H), 4.31 (td, J = 6.2, 4.6 Hz, 4H), 3.88-3.79 (m, 4H), 3.47 (s, 6H). ¹³C NMR (75 MHz, CDCl₃): δ 153.16, 151.90, 142.69, 117.32, 115.55, 109.62, 100.85, 70.51, 70.45, 69.61, 69.42, 59.38.

6,7-bis(2-methoxyethoxy)quinazolin-4(3H)-one (5)
After dissolving compound 4 (0.4 g, 1.35 mmol) and InCl₃ (0.3 g, 1.35 mmol) in formamide (20 mL), the mixture was put into microwave reactor to react under a power of 400 W for 1 h at 110 °C. The mixture was then filtered and washed with brine. The filtrate was extracted by ethyl acetate and organic layer was collected. After concentration followed by recrystallization in ethyl acetate, compound 5 of white solid was obtained (0.2 g, 50%). ESI-MS m/z: 295.3 [M+H]⁺, 317.3 [M+Na]⁺. ¹H NMR (300 MHz, CDCl₃): δ 12.17 (s, 1H), 8.04 (s, 1H), 7.51 (s, 1H), 7.09 (s, 1H), 4.36-4.14 (m, 4H), 3.83 (s, 4H), 3.45 (d, J = 0.7 Hz, 6H). ¹³C NMR (75 MHz, CDCl₃): δ 162.39, 154.74, 148.62, 145.33, 142.76, 115.65, 109.06, 106.47, 70.63, 70.48, 68.50, 68.43, 59.27, 59.23.

RESULTS AND DISCUSSION
Commonly, quinazolinone was produced from substituted benzonitrile through a scheme of at least two steps14,15, and the yield ranged 8%~30%10,11,16. We firstly used this process (Scheme 2) to synthesize compound 5. Briefly, the three steps of this reaction needed 13 hours to finish, including reduction of nitrobenzotride intermediate to give aminated benzonitrile one, then converting of cyano group into an amide group and finally formation of quinazolinone ring by conventional Niementowski cyclization15. The results showed that the yield of heterocyclization of quinazolinone ring was 29.8%, which was similarly low as those reported16 and would lead a low overall yield thereafter. In conclusion, this reaction route was somewhat complicated and time-consuming.
Scheme 2. Reagents and conditions: (a) ammonium formate, 5% Pd/C, 77 °C, 2 h; (b) K₂CO₃, DMSO, 30% H₂O₂, 0°C to rt. 1 h; (c) Formamid, formic acid, r.t. to 100°C, 10 h.

There have been many researchers dedicating to optimize the reaction route and improve the yield of quinazolinone intermediate. Kundu et al. synthesized some antitumor quinazolone precursors by one-pot reductive cyclization method intending to increase the yield of heterocyclization. The structure and corresponding yield of the investigated quinazolinone derivatives were listed in Fig. 2. As shown in Fig. 2, when there were no substituents at the meta and para positions on the benzene ring, the yield of the cyclization reaction was the highest about 90%. When there was substituent at meta position, the synthesis yield of quinazolinone was reduced. However, the reaction lasted at least five hours at 150 °C. In another study of synthesizing quinazoline intermediates for developing cannabinoid receptor agonists, Raimo Saari et al. successfully produced quinazolin-4(3H)-one using 2-aminobenzonitrile as starting material by microwave-assisted reaction (100 °C) within 5 minutes and got a yield of 64%. Besides, Li Feng et al. also developed a method to yield compound 5 by microwave reaction using substituted aminobenzamide as starting material. The heterocyclization was realized in the presence of iridium complex and ambient of N₂ within 2 hours at 120 °C, and the yield of cyclization was 85%. However, this reaction was just the last step of the commonly used three-step scheme for synthesis of compound 5. The results encouraged us to figure out whether the one-pot microwave reaction could improve the synthesis of compound 5.

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Figure 2 The structure and yield of quinazolinone derivatives prepared by one-pot reductive cyclization reported by Kundu et al.12.

Five different conditions of microwave reaction were investigated in this work. The effect of microwave power, temperature and time of reaction on the yield of compound 5 was listed in Table 1. The results showed that the combination of high microwave power or/and reaction temperature and long reacting time were unfavorable to the synthesis, which yield no or just a
bit of product. When using moderate power, reaction temperature and time, the yield of 50% was achieved, which was 1.68 folds as much as that got by the commonly used three-step synthetic scheme. More significantly, only one step was needed to produce quinazolinone derivatives from substituted nitrobenzonitrile in 1 hour, making the synthesis friendlier.

Table 1 Results of condition screening of microwave reaction in synthesis of 6,7-bis(2-methoxyethoxy)quinazolin-4(3H)-one.

<table>
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CONCLUSIONS

In our study, compound 5, the important intermediate in synthesis of erlotinib analogues, was successfully produced by using an easy one-pot microwave reaction from starting material of substituted nitrobenzonitrile. and the reaction time was only one thirteenth of the commonly used three-step synthetic scheme. Although the two substituents on benzene ring of the starting material were somewhat bulky, a relatively satisfactory yield was achieved under appropriated reaction condition, which would further ensure an acceptable overall yield of erlotinib analogues designed. Therefore, the results of this work may give valuable reference to synthesis of other quinazolinone derivatives of different substituents.

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