Synthesis Of Novel Thiophene-Bipyrimidine EGFR Inhibitors

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Abstract. Pyrimidine is a prevalent component in numerous antineoplastic medications. Herein, starting with Olmutinib, by applying the concept of skeleton transition, the thiopheno [3,2-d] pyrimidines were categorized into a thiophene bipyrimidine framework as the foundation, leading to the creation of a novel thiophene bipyrimidine compound. The target product was synthesized through nucleophilic substitution, Suzuki coupling, and reduction reactions employing 2-pyrrolidine-4-trichloropyrimidine as the raw material and triethylamine as the base, leading to the efficient synthesis of compound 7.The structure of compound 7 was confirmed by MS and 1H NMR spectrum and the total yield was high up to 65.7%.

Keywords: Novel thiophene bipyrimidines; EGFR inhibitors; synthesis.

1. Introduction

Cancer is the leading cause of human mortality. The number of cancer deaths in China in 2022 is 3.21 million, representing 30% of all worldwide cancer fatalities[1]. The most common cancer is lung cancer, responsible for 26.8% of all cancer fatalities[2]. Non-small cell lung cancer (NSCLC) is a significant factor in the realm of lung cancer [3]. According to the treatment guidelines of the national cancer prevention and control platform for lung cancer patients, early detection of lung cancer is crucial for effective treatment. The majority of early-stage lung cancers can be effectively controlled with treatment[4]. For patients with early-stage non-small cell lung cancer, our goal is to prioritize surgical resection for optimal curative outcomes. Treatment emphasis should be on swift intervention at this stage. Timely detection leads to quicker treatment response and increases the likelihood of a successful cure[5]. Targeted drug therapy is currently a prominent research focus due to the specificity of targeted drugs [6]. In recent research, elevated or abnormal EGFR expression has been detected in numerous solid tumors. EGFR is associated with tumor cell proliferation, angiogenesis, tumor invasion, metastasis, and inhibition of apoptosis[7, 8]. EGFR plays a key role in the onset and progression of various cancers due to mutations or gene amplification, particularly in NSCLC, CRC, and etc[9]. Based on the study of the structure, pathway, and mechanism of EGFR, researchers have discovered that small molecule targeted drugs are more effective than chemotherapy and cause less harm to patients. Small molecule targeted drugs are created to target precise cancer sites at the molecular level. These drugs can inhibit the proliferation, differentiation, and migration of cancer cells by blocking signal transduction processes[10]. Additionally, they can impede the formation of tumor blood vessels[11] and hinder tumor cell growth[12] by restricting the supply of nutrients to the tumor.

Several potent small molecule inhibitors targeting EGFR have been developed and implemented in clinical settings. For instance, Rociletinib (CO-1686) is an oral EGFR inhibitor capable of suppressing the activity of EGFRL858R/T790M and EGFRWT. Pazopanib (GW786034) is a novel multi-target inhibitor of VEGFR1, VEGFR2, VEGFR3, and PDGFR. Imatinib (STI571) serves as a multi-target tyrosine kinase inhibitor. Dasatinib (BMS-354825) is a promising multi-target inhibitor known to trigger autophagy and apoptosis, exhibiting anti-tumor properties against Abl, Src, and cmurKitDasatinib. The molecular structures of these compounds are illustrated in Figure 1.

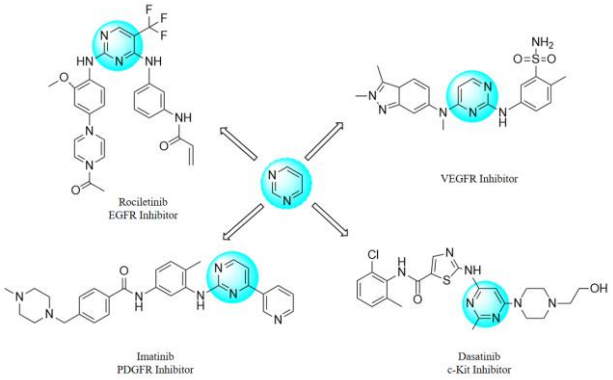

Fig. 1 Inhibitors containing pyrimidine structure

Given the promising future of the pyrimidine structure in targeted anti-tumor applications, we have made modifications to the original compound by transitioning from thienopyrimidine of Olmutinib to thiophene bipyrimidine. This adjustment holds significant relevance for the synthesis of similar antineoplastic drugs. The establishment of an efficient synthesis method for these new thiophene bipyrimidine compounds is detailed in this paper.

2. Materials and methods

^{1}HNMR spectra were performed using Bruker 400 MHz spectrometers (Bruker Bioscience, Billerica, MA, USA) with TMS 210 as an internal standard. Mass spectra (MS) were taken in ESI mode on Agilent 1100 LC-MS (Agilent, Palo Alto, CA, USA). All the materials were obtained from commercial suppliers and used without purification, unless otherwise specified. Yields were not optimized. TLC analysis was carried out on silica gel plates GF254 (Qingdao Haiyang Chemical, China).

3. Synthesis of compounds

The structures and the synthetic route were shown in Figure 2.

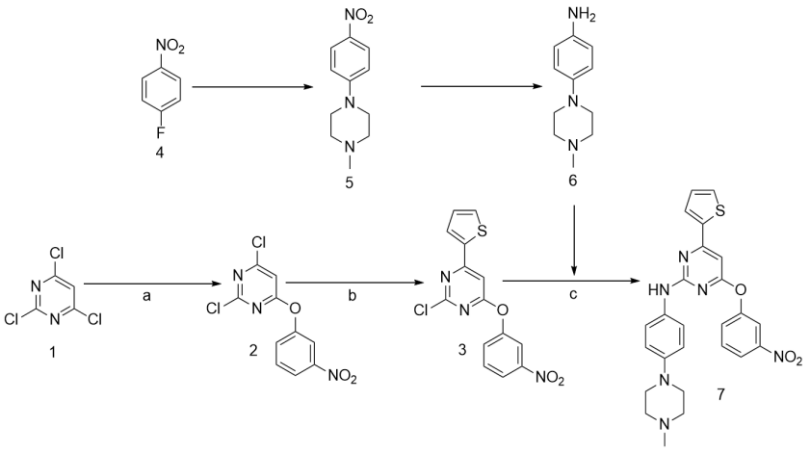

Fig 2. The synthetic route of compound **7**

3.1. Reagents and conditions

(a) Trichloro pyrimidine, M-nitrophenol, Triethylamine, Isopropanol, 4 h, r.t.; (b) 2-thiophene boric acid, K2CO3, Pd(PPh3)Cl2, DCE:H2O(5:1), 80 oC, 6 h; (c) 1-Methylpiperazine, DMSO, 120 oC, 3 h; (d) Zn powder, HCl, AcOH, 80 ℃, 6 h; (e) Secondary amines, Trifluoroacetic acid, Dioxane, 100 ℃, 8 h.

3.2. Preparation of intermediate 2,4-dichloro-6-(3-nitrophenoxy)pyrimidine (2)

2,4,6-trichloropyrimidine (1) (5.0 g, 0.030 mol) was dissolved in a sufficient amount of isopropanol (100 mL) in a round-bottom flask. Then mix with p-nitrophenol (4.0 g, 0.031 mol) and triethylamine (6.0 g, 0.060 mol) in a proper amount of isopropanol and add it to the round-bottom flask. The mixture was stirred at room temperature for 4 h, monitoring the reaction with TLC during the process. After the reaction is complete, evaporate the organic solvent under reduced pressure, add water (50 mL) to the flask, sonicate and filter to remove water. The resulting filter cake is dried to obtain intermediate 2 (white solid, yield: 95.2%).1H NMR (400 MHz, DMSO-d6) δ 8.23 (d, J = 11.3 Hz, 2H), 7.81 (d, J = 4.7 Hz, 2H), 7.62 (s, 1H).

3.3. Preparation of intermediate 2-chloro-4-(3-nitrophenoxy)-6-(thiophene-2-yl) pyrimidine (3)

To a stirred mixture of the intermediates 2 (2.0g, 0.007mol), 2-thiophene boric acid (1.0g, 0.008mol), and K2CO3 in a proper amount of mixed solvent (ethylene glycol dimethyl ether: water = 4:1, 25ml), Pd (PPh3)Cl2 catalyst was added to the mixture and stirred at 80 °C for 6 h, protected by nitrogen. During the process, the reaction was monitored by TLC. Upon completion, the crude product was concentrated under reduced pressure. The crude product was then purified by column chromatography on silica. Intermediate 3 was obtained by using dichloromethane/methanol (v/v, from 100:1 to 40:1 as eluent). White solid; yield: 49.5%;1H NMR (400 MHz, DMSO-d6) δ 8.21 (dd, J = 10.3, 7.3 Hz, 3H), 7.94 (d, J = 4.9 Hz, 1H), 7.85 – 7.78 (m, 3H), 7.29 (t, J = 4.4 Hz, 1H).

3.4. Preparation of intermediate 1-methyl-4-(4-nitrophenyl) piperazine (5)

The preparation process is as follows: dissolve the commercially available p-fluoronitrobenzene (4) (5.0g 40mL 0.035mol) in a round bottom flask, then add 1-methylpiperazine (4.0g 40mL 0.040mol) into the round bottom flask, stir at 120 oC for 4 h, monitor the reaction during the process, add water (50 mL) to the flask after ultrasonic dispersion, and remove the water. The intermediate 5 was prepared by drying the filter cake. Yellow solid; yield: 92.7%;1H NMR (400 MHz, CDCl3) δ 8.11 (d, J = 9.0 Hz, 2H), 6.82 (d, J = 9.0 Hz, 2H), 3.43 (t, J = 5.1 Hz, 4H), 2.55 (t, J = 5.1 Hz, 4H), 2.35 (s, 3H).

3.5. Preparation of intermediate 4-(4-methylpiperazine-1-yl) aniline (6)

Intermediate 5 (5.0g 40mL 0.023mol) was dispersed in a round bottom flask, and hydrochloric acid solution was dropwised, then zinc powder (4.5g score 0.069mol) was slowly added to the mixture and reacted at 80 °C for 6h. After the reaction was over, zinc powder was removed by filtration, water and organic solvents were removed by vacuum concentration, and pure intermediate 6 was obtained by recrystallization with ethyl acetate/petroleum ether. Grey solid; yield: 85.7%;1H NMR (400 MHz, DMSO-d6) δ 6.67 (d, J = 8.3 Hz, 2H), 6.48 (d, J = 8.2 Hz, 2H), 4.55 (s, 2H), 2.89 (t, J = 4.9 Hz, 4H), 2.41 (t, J = 4.9 Hz, 4H), 2.19 (s, 3H).

3.6. Preparation of intermediate N-(4-(4-methylpiperazine-1-yl) phenyl)-4-(3-nitrophenoxy)-6-(thiophene-2-yl) pyrimidine-2-amine (7)

To a solution of Intermediate 3 (5.0 g, 0.015 mol) and Intermediate 6 (5.0 g, 0.025 mol) in dioxane (100 mL), add trifluoroacetic acid (5 mL). Heat the mixture at 100 °C for 12 h. Filter the reaction solution, spin the filtrate, and extract with DCM and water. Remove water with anhydrous sodium sulfate, reduce pressure, concentrate the solvent, and add $NaHCO_3$ under ultrasound. Remove $NaHCO_3$ powder by filtration and concentrate under reduced pressure. Add isopropanol by ultrasonic until oil is dispersed. Obtain buff filter cake by filtration, and dry to obtain intermediate 7. Buff solid; yield: 65.7%;^{1}H NMR (400 MHz, $CDCl_3$) δ 8.20 – 8.13 (m, 2H), 7.75 (d, *J* = 3.8 Hz, 1H), 7.63 (t, *J* = 8.0 Hz, 1H), 7.60 – 7.56 (m, 1H), 7.54 (d, *J* = 5.0 Hz, 1H), 7.36 (s, 2H), 7.21 –7.17 (m, 1H), 6.94 (s, 1H), 6.82 (s, 2H), 6.71 (s, 1H), 3.24 – 3.18 (m, 4H), 2.72 (s, 4H), 2.45 (s, 3H).

4. Conclusions

Using 2,4,6-trichloropyrimidine (1) as a starting material, a new thieno [2,3-*b*] pyridine-containing compound *N*-(4-(4-methylpiperazin-1-yl)phenyl)-4-(3-nitrophenoxy)-6-(thiophen-2-yl)pyrimidine-2-amine was synthesized through several steps including nucleophilic substitution reactions, Suzuki coupling reactions, and reduction reactions. This synthesis method is convenient and quick, with a highly yield of up to 65.7%. The structure of compound 7 was confirmed by MS and ^{1}H NMR spectra.

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