Synthesis of 2-chloro-4-(3-nitrophenoxy)-6-(thiophen-2-yl) pyrimidine

Jianlan Kou [1, *], Feiyi Yang [2]

[1] Teaching and Research Department of Mathematics and Chemistry, Nanchang Health School, Nanchang 330006, People's Republic of China

[2] School of pharmacy, Jiangxi Science & Technology Normal University, Nanchang 330013, China

* Corresponding author Email: 1547589046@qq.com

Abstract. 2-Chloro-4-(3-nitrophenoxy)-6-(thiophen-2-yl) pyrimidine (c) is an important intermediate of small molecule anticancer drugs. In this paper, a rapid synthesis method for the target compound has been developed. Compound c was synthesized from 2,4,6-trichloropyrimidine (a) through two steps including nucleophilic substitution and coupling reaction. The structure of the target compound c was confirmed by 1H NMR and MS spectrum. Furthermore, the synthetic method was optimized. The total yield of the two steps was up to 44.6%.

Keywords: 2-Chloro-4-(3-nitrophenoxy)-6-(thiophen-2-yl) pyrimidine, Synthesis.

1. Introduction

Malignant growth of tumor will destroy normal tissues and organs, and transfer to other parts of the body, resulting in serious organ function damage, and then form cancer, which is a serious threat to human health [1-2]. According to the rising incidence rate and mortality rate of cancer, IARC predicts that the new cancer cases and cancer deaths will be 18 million 100 thousand (without melanoma skin cancer) and 9 million 600 thousand (without melanoma skin cancer) [3]. Lung cancer has the highest number of new cases and deaths among all cancers worldwide [4]. Lung cancer is divided into two types: non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC). Specifically, NSCLC is known as a type of lung cancer and accounts for about 80-85% of all lung cancers [5]. Approximately 10%-50% of NSCLC patients with epidermal growth factor receptor (EGFR) activating mutations, such as in-frame deletions in exon 19 (Ex19del) or missense mutations in exon 21 (L858R) [6]. NSCLC is mainly treated by a combination of surgical procedures, but 80% of lung cancer patients have already lost the opportunity of surgery when they are clearly diagnosed, and only about 20% of patients can be treated surgically. The current 5-year survival rate of surgery is around 30% with unsatisfactory results, while nearly half of the patients will develop local recurrence or distant metastases within a short time after surgery. The survival of retreatment after failure of platinum-based combination chemotherapy is only 5-7 months, and the level of chemotherapy for advanced lung cancer has reached a bottleneck. Chemotherapy and radiotherapy can kill cancer cells on a large scale, but the recovery rate is low because they can't distinguish tumor cells from normal cells. On the other hand, traditional chemotherapeutic drugs lack selectivity between normal and tumor cells, have strong toxic side effects, and are prone to drug resistance and other disadvantages. In addition, many cancers have developed resistance to above therapies, which makes the next treatment ineffective [7]. It can be seen that lung cancer is a great threat to human health and there is an urgent need to find effective drugs and methods to treat lung cancer.

In recent years, with the advancement of molecular biology technology and further understanding of the relationship between cell receptors and molecules that regulate proliferation in tumor pathogenesis, people have started to carry out treatments that target cell receptors, regulatory molecules and key genes, called targeted therapies. Unlike traditional cytotoxic drugs, molecularly targeted therapeutic drugs take cell receptors, genes, regulatory molecules and other signaling as their targets and act specifically on specific sites of tumor cells to kill tumor cells in a highly selective manner with high safety, good tolerability and mild toxic side effects, thus having very great advantages and application prospects and opening up a new field in tumor treatment. In the early 21st

century, NSCLC was classified into molecular phenotypes according to various molecular markers, and new drugs were invented based on the driver genes related to tumorigenesis and development as research targets for more targeted and individualized molecular targeting therapy, and since then, the treatment of NSCLC has started the path of precise therapy with genes as targets. The EGFR is one of the most studied of the many driver genes.

Protein Tyrosine Kinases (PTKs) are one of the key factors in the cell signaling process, which are closely related to cell proliferation, differentiation, apoptosis, and metabolism [8]. EGFR is the earliest tyrosine kinase reported in the literature and is a member of the ErbB family of receptor tyrosine kinases, which are cell membrane glycoproteins with a molecular weight of 170 kDa [9]. It is the expression product of the proto-oncogene HER-1, which is localized on the cell membrane, mainly in epithelial cells, mesenchymal cells and neuronal cells. Its structure includes: a glycosylated extracellular region (extracellular ligand-binding region), a single-stranded hydrophobic transmembrane region and a protein kinase active region [10]. EGFR plays a crucial role in the regulation of cell proliferation, apoptosis and differentiation, and its activation is achieved through ligand binding. EGFR tyrosine kinase is an important hub for extracellular signaling into the cell, and it plays an important role in signaling, cell proliferation, differentiation, and various regulatory mechanisms, and abnormal EGFR signaling has been found to be closely associated with tumor formation [11]. Due to the essential role of the EGFR family in cancer formation, the EGFR tyrosine kinase family has become the target of choice for targeted cancer therapy, especially for NSCLC [12-14]. Current investigations have shown that down-regulation of EGFR tyrosine kinase phosphorylation expression levels can inhibit tumor growth. By analyzing the structural characteristics of EGFR and selecting their specific sites as targets for interfering with signaling, it has become a new idea for targeted antitumor drug development. [15]. The structure of EGFR was shown in Fig.1.

Recently, tremendous efforts have been made to develop new treatments aimed at improving the specific targeting of cancer cells and overcoming resistance to current therapies. Many drugs targeting EGFR have been developed at this stage, including monoclonal antibodies, small molecule tyrosine kinase inhibitors, antisense oligonucleotides [16], and antimycin conjugates [17]. Among them, monoclonal antibodies and small molecule tyrosine kinase inhibitors are the most promising and widely used EGFR inhibitors. Tyrosine kinases play an extremely important role in the signaling of growth factors. Activation of tyrosine kinases can lead to tumor cell proliferation, anti-apoptosis, angiogenesis and metastasis of cancer. Therefore, tyrosine kinase is a key target of EGFR. Small molecule EGFR tyrosine kinase inhibitors (EGFR TKIs) selectively target the catalytic region of intracellular tyrosine kinases, competing with ATP to bind the kinase pocket, thereby inhibiting tyrosine phosphorylation and interrupting kinase catalysis-induced downstream signaling. Although the first generation of reversible EGFR inhibitors have been clinically more successful, the emergence of drug resistance, especially due to T790M mutations, has reduced efficacy. To overcome drug resistance, researchers have developed irreversible inhibitors (second generation EGFR TKIs) that contain Michael receptors that covalently bond to cysteine residues (Cys797) located at the entrance of the ATP binding pocket, such as Afatinib, Dacomitinib [18-19]. Afatinib was the first irreversible second-generation EGFR inhibitor approved by the FDA, but more serious side effects such as rash and gastrointestinal toxicity were observed after administration. To reduce the toxic side effects, researchers went on to develop third-generation EGFR inhibitors, such as AZD9291 and CO-1686 [20-21]. Therefore, the development of tyrosine kinase inhibitors for patients with EGFR mutations is seen as an important part of the treatment of NSCLC. Among them, compound c is a significant intermediate for the synthesis of epidermal growth factor tyrosine kinase inhibitors. Hence, design and synthesis of compound c derivative as small molecule inhibitors played an essential role in the study of anti-non-small cell lung cancer. Many compound c derivatives which exhibited potential biological activities, such as N-(3 -((2- ((4-(4-methylpiperazin-1-yl)phenyl)amino) thieno[3,2-d] pyrimidin-4-yl)oxy)phenyl)acrylamide(1),N-(3-((2-((3-cyanophenyl)amino)-6-(thiophen-2yl) yrimidin-4-yl)oxy)phenyl)-3-methylbut-2enamide(2),(E)-4-(dimethylamino)-N-(3-((2-((1-methyl-

1H-pyrazol-3-yl)amino)-6-(thiophen-2-yl)pyrimidin-4yl)oxy)phenyl)but-2-enamide(3),(Z)-N-(3-((4-(2,3-dihydro-4H-1,4-oxazin-4-yl)-6-(5-((dimethylamino)methyl)thiophen-2-yl)-1,3,5-triazin-2-yl)oxy)phenyl)but-2-enamide(4), were shown in Fig. 2. [22-24].

Most of the synthetic methods of compound c which reported in the literature have the drawbacks, such as lower yield [23]. Compound a is a key intermediate the synthesis of 2-chloro-4-(3-nitrophenoxy)-6-(thiophen-2-yl) pyrimidine (c). The synthesis of the target compound is necessary. The structure of compound c was shown in Fig. 3.

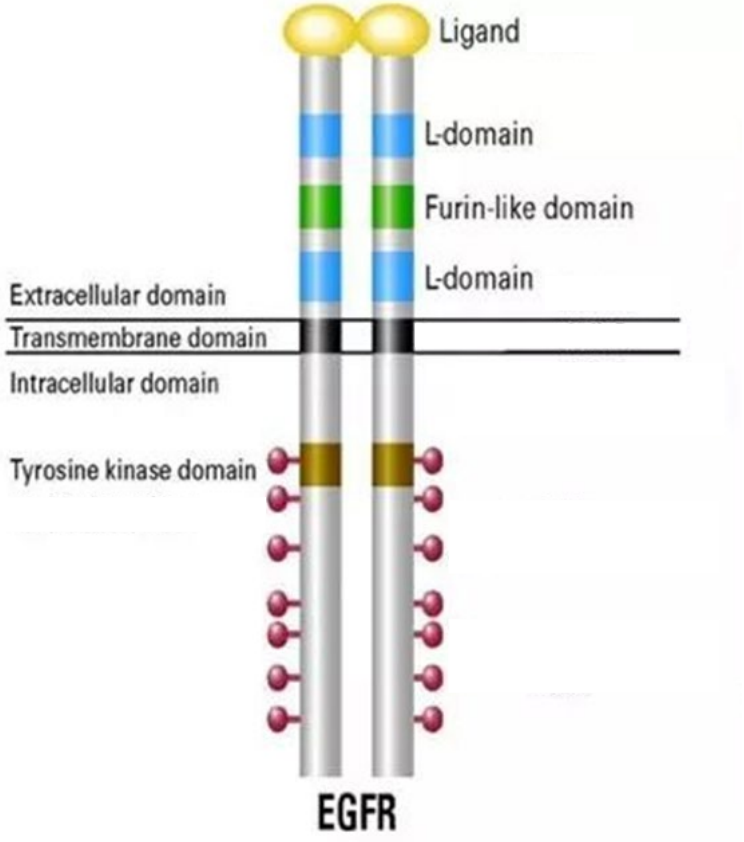


Fig. 1 Structure of EGFR

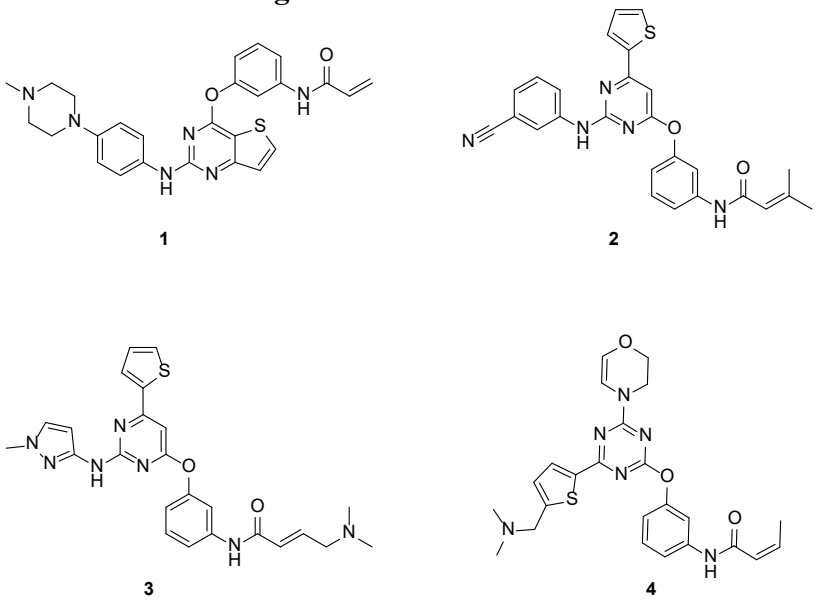
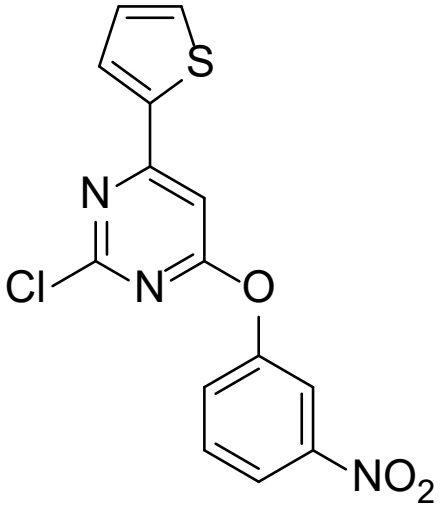


Fig. 2 Structures of the active compounds containing phenoxypyrimidine



c

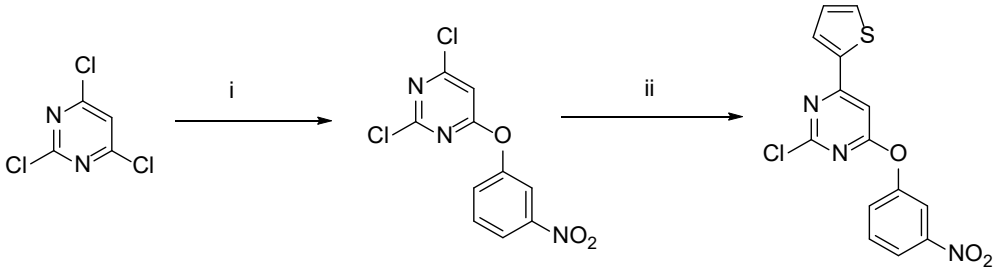
Fig. 3 Structure of the target compound c

2. Materials and methods

Nuclear magnetic resonance spectroscopy was performed using a Bruker 400 MHz spectrometer (Bruker Bioscience, Billerica, MA, USA) with TMS 210 as internal standard. Mass spectrometry (MS) was taken in ESI mode on Agilent 1100 LC–MS (Agilent, Pato, CA, USA). All materials were obtained from commercial suppliers and used without purification, unless otherwise specified. The yields were not optimized. TLC analysis was performed on silica gel plates GF254 (Qingdao Ocean Chemical, China).

3. Synthesis of compounds

The structures and the synthetic route of compounds were shown in Fig. 4.



a **b** **c**

Fig. 4 The synthetic route of compound c

Reagents and conditions:

(i) 2,4,6-Trichloropyrimidine, M-nitrophenol, Triethylamine, Isopropanol, 4 h, r.t.

(ii) 2-Thiophene boric acid, K2CO3, Pd (PPh3) Cl2, 1,2-Dimethoxyethane: H2O (5:1), 80 ℃, 6 h.

3.1 Preparation for 2,4-dichloro-6-(3-nitrophenoxy) pyrimidine (b)

Compound a (5.0 g, 27 mmol) was dissolved in an appropriate amount of isopropanol (15mL) in a round bottom flask, then m-nitrophenol (4.0 g, 29 mmol) and triethylamine (6.0 g, 59 mmol) were mixed in isopropanol (25 mL) and slowly added to the round bottom flask containing 2,4,6-trichloropyrimidine. And the mixture was stirred at room temperature for 4 hours, and then the reaction was monitored by TLC analysis. After the reaction, the solvent is concentrated under reduced pressure and then pumped by adding a large amount of water. The resulting filter cake was dried to obtain compound b as a white powdered solid in 93% yield (7.45 g). MS (ESI): m/z [M+H] + 285.9.

3.2 Preparation for 2-chloro-4-(3-nitrophenoxy)-6-(thiophen-2-yl) pyrimidine (c)

Compound b (2.0 g, 7 mmol), 2-thiophenylboric acid (1.0 g, 8 mmol) and K2CO3 (0.9 g, 7 mmol) were dissolved in a mixture solvent of 1,2-dimethoxyethane (15 mL) and water (3 mL), then the Pd catalyst was added to the mixture, protected by a stream of nitrogen and stirred at 80 °C. After being stirred for 5 h, the reaction mixture was quenched with H2O and extracted with DCM (50 mL x 3). The combined organic layer was dried over anhydrous Na2SO4 and concentrated under reduced pressure. The residue was purified by silica gel chromatography (EtOAc: petroleum ether 1:4) to give compound c as a white powdered solid in 48% yield (1.12 g). MS (ESI): m/z [M+H] + 334.0. 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).

4. Conclusions

In conclusion, 2-chloro-4-(3-nitrophenoxy)-6-(thiophen-2-yl) pyrimidine (c) was synthesized from the commercially available 2,4,6-trichloropyrimidine (a) and m-nitrophenol through two steps of nucleophilic substitution and coupling reaction. The synthesis of compound c was optimised by changing the reaction conditions, resulting in an improved yield. Its structure was confirmed by 1H NMR spectrum. Thus, this synthetic method can be used for the synthesis of derivatives of compound c.

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