# Synthesis of 4, 6-dichloro-1H-pyrazolo [3, 4-d] pyrimidine

**Jie He, Zhihui Zhou, Bing Wang, Pengwu Zheng, Shan Xu***

School of Pharmacy, Jiangxi Science & Technology Normal University, Nanchang 330013, China

*Corresponding author: shanxu9891@126.com

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**Abstract:** In recent years, 2, 4-dichloropyrimidine derivatives have found wide application in drug design. In this work, a rapid synthetic method for target compounds was established. Compound (3**)** was synthesized from 5-amino-1*H*-pyrazole-4-carboxamide and urea through two steps including cyclization and chlorination. The structure of the target compound was confirmed by $^{1}$H NMR and MS spectrum. Furthermore, the synthetic method was optimized. The total yield of the two steps was 49.5%.

## 1. Introduction:

It has been found that the Epidermal Growth Factor Receptor (EGFR) signaling pathway plays an important role in tumorigenesis and regulates many important biological processes in tumorigenesis and development, which including promoting cell proliferation, differentiation, and protein synthesis, metastasis, inhibiting cell apoptosis and promote angiogenesis [1-5]. Hence, inhibition of this signaling pathway has become a hot spot for tumor prevention and treatment, attracting the attention of many research institutions and researchers.

The first generation EGFR tyrosine kinase inhibitors (TKIs) have achieved great success in clinical, such as Gefitinib [6] and Erlotinib [7]. However, the emergence of drug resistance, especially the resistance caused by mutations of T790M and L858R, has reduced the efficacy of tumor patients. In order to overcome the drug resistance brought by the first generation EGFR inhibitors, scientific researchers have developed a second generation irreversible EGFR inhibitor, which contains Michael receptors and can be located at the entrance of the ATP binding pocket. The cysteine amino acid residue (Cys797) is covalently bonded, which can improve the time of drug effect and the actual efficacy, like Afatinib [8], Dacomitinib [9] and so on. Afatinib is a representative drug of the irreversible second generation EGFR inhibitors, but some serious side effects, such as rashes and gastrointestinal toxicity, have appeared after the medication. Furthermore, in order to reduce the side effects of second generation EGFR inhibitors, researchers have further developed third generation EGFR inhibitors, for example, Osimertinib [10] and CO-1686 [11]. Unfortunately, new resistance still appears inevitably.

As treatment resistances arises frequently, it is particularly important to find a way to get rid of clinical difficulties, prevent or delay the EGFR-TKIs resistance or develop new EGFR-TKIs drugs with good tumor selectivity, high efficiency and safety. In the last ten years, there were many small molecule anticancer drugs had been reported, and they are classified as pyrimidine, quinazoline or pyrrole, etc. Among them many molecules contained the 2, 4-dichloropyrimidine (1). Therefore, design and synthesis of 2, 4-dichloropyrimidine (1) derivative as small molecule inhibitors played a great role in the study of anticancer drugs. The structures of these compounds were shown in Fig.1. For example, N-(2-methoxy-4- (4-methylpiperazin-1-yl)phenyl) -5-methyl-4- (3-nitrophenoxy) pyrimidin-2-amine (2) [12-13], N-(2-((2-(dimethylamino) ethyl) (methyl)amino)-4-methoxy-5-((4-(1-methyl-1*H*-indol-3-yl)pyrimidin-2-yl)amino) phenyl)acrylamide (3) [14-16], N-(3-((2-((4-(4-methylpiperazin-1-yl)phenyl)amino)thieno[3,2-*d*]pyrimidin-4-yl)methyl)phenyl)acrylamide (4) [17-18].

The Structures of the intermediate active compounds were shown in Fig. 1

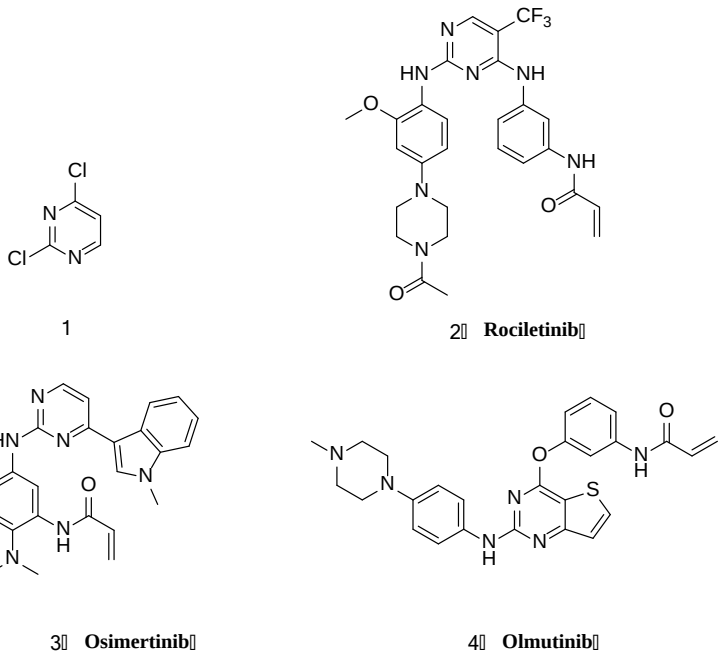


Fig 1. Structures of the intermediate active compounds containing the intermediate.

## 2. Materials and methods

NMR 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 (Qindao Haiyang Chemical, China).

## 3. Synthesis of compounds

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

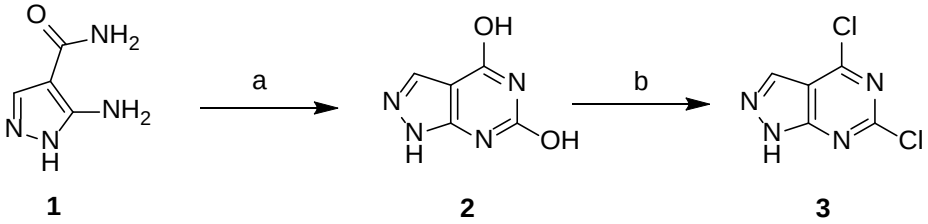


Fig 2. The synthetic route of compound 3.

Reagents and conditions: (a) urea, 190℃, 2 h; (b) $POCl_3$, 110 ℃ reflux, 4 h.

### 3.1 Preparation for 1*H*-pyrazolo [3, 4-*d*] pyrimidine-4, 6-diol (2)

5-amino-1*H*-pyrazole-4-carboxylic acid amide **(1)** (5 g, 0.0396 mol) and urea (23.6 g, 0.3937 mol) were heated together at 190℃ for 2 h. The solution became turbid gradually, and the reaction was monitored by TLC. After the reaction, add 10% KOH solution(10 g of KOH in 90 mL of water) first, then carefully add dilute hydrochloric acid for acidification( pH = 4-5), finally sonicate and suction filter to obtain the white solid (4.5 g, 75%). $^1$H NMR (400 MHz, DMSO-$d_6$) δ 13.29 (s, 1H), 11.34 (s, 1H), 10.67 (s, 1H), 8.33 (s, 1H).

### 3.2 Preparation for 4, 6-dichloro-1*H*-pyrazolo [3, 4-*d*] pyrimidine (3)

A mixture of 1*H*-pyrazolo[3,4-*d*]pyrimidine-4,6-diol (2) (3 g, 19.7 mmol), $POCl_3$ (20 mL) and DMF (6 d) was heated and stirred for 6 h at 110℃, and the reaction was monitored by TLC. The mixture was concentrated under reduced pressure to afford product as viscous oil. Then, the mixture was transferred to a beaker; ice water acetate was added slowly with stirring. Filtration, the filter cake was washed with ice-water, dried to obtain a yellow solid (2.45 g, 66%).

## 4. Conclusions

In general, 4, 6-dichloro-1*H*-pyrazolo [3, 4-*d*] pyrimidine (3) was optimized by two steps including cyclization and chlorination. Due to optimization of the synthesis conditions of the target compound 3, the purity of the product was higher. Its structure was confirmed by $^1$H NMR spectrum.

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