Synthesis of 3, 8-dichloro-[1, 2, 4] triazolo [4, 3-a] pyrazine

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Abstract. 3, 8-dichloro-[1, 2, 4] triazolo [4, 3-a] pyrazine is an important intermediate for the synthesis of many small molecule anticancer drugs. It was synthesized from 2, 3-dichloropyrazine, N₂H₄•H₂O (80%) and 2, 2, 2-trifluoroacetic anhydride through four steps including substitution, acylation, cyclization and chlorination reactions. The structure were confirmed by MS and ¹H NMR. Furthermore, the synthetic method was optimized. The total yield of the four steps were 76.57%.

1. Introduction
Cancer is one of the most serious diseases that threaten human life. As we all know, cancer become more and more complex, the traditional treatment methods such as surgery, chemotherapy and radiotherapy is not enough to treat completely [1]. The emergence of small molecule targeted inhibitors has made new progress in the research of anticancer drugs. Small molecule target inhibitors mainly act on the signaling pathway of cancer cell growth, further hindering cancer cell growth and promoting apoptosis [2, 3]. 3, 8-dichloro-[1, 2, 4] triazolo [4, 3-a] pyrazine (1) are important intermediates for many small molecule inhibitors. The discovery of these intermediates promotes the development of anti-tumor drugs, and these intermediates have good pharmacokinetic activity [4-6]. There are many signaling pathways in our body that affect the growth, metastasis, proliferation and proliferation of cancer cells. Small molecule inhibitors can inhibit cancer cell growth and promote apoptosis by binding to signaling pathways [7-10].

In recent years, many small molecule anticancer drugs have been reported, and a large number of bioactive compounds have been designed and synthesized. Among them, 8-chloro-3-(trifluoromethyl)-[1, 2, 4]triazolo[4,3-a]pyrazine (1), 6-chloro-9H-purin-2-amine (2) and 7-chloro-5-(trifluoromethyl)-[1, 2, 4]triazolo[1,5-a]pyrimidine (3) are important intermediates, which show potential biological activity and play a crucial role in the study of anticancer drugs. Fig. 1 shows the structure of representative compounds. The synthesis of 8-chloro-3-(trifluoromethyl)-[1, 2, 4] triazolo [4, 3-a] pyrazine (1) as a key intermediate has the disadvantage of low yield in many reports. Therefore, this paper mainly optimizes the synthetic route of 8-chloro-3-(trifluoromethyl)-[1, 2, 4] triazolo [4, 3-a] pyrazine (1). By optimizing the reaction conditions, the production cost was reduced and the production rate was increased.
2. Materials and Methods
NMR spectra were performed using Bruker 400 MHz spectrometers with TMS 210 as an internal standard. Mass spectra (MS) were taken in ESI mode on Agilent 1100 LC–MS. All the materials were obtained from commercial suppliers and used without purification, unless otherwise specified. Yields were optimized. TLC analysis was carried out on silica gel plates GF254.

3. Synthesis of Compounds
The structures and the synthetic route were shown in Scheme 1.

**Scheme 1.** The synthetic route of compounds 1

Reagents and conditions: (a) N$_2$H$_4$·H$_2$O (80%), ethanol, 85°C, reflux, 2 h; (b) 2,2,2-trifluoroacetic anhydride, ice bath stirring, 2.5 h; (c) PPA, 100°C, reflux, 4 h; (d) POCl$_3$, DMF, 80°C, after 0.5 h switch to 100°C, reflux, 2 h.

4. Preparation of Compound 5
2, 3-dichloropyrazine (5.0 g, 0.134 mol) in flask, directly with ethanol (20 mL) as solvent, added a small amount of hydrazine hydrate (5.24 g, 0.42 mol) at a time and added it several times, the reaction is carried out under the 85°C and the reflux device, stirring for 2 h. After cooling, the reaction liquid is added to ice water and stirred and filtered to obtain a yellowish crystal product (compound 5) (4.85 g, 97%). ESI-MS m/z: [M+H]$^+$ 145.02, 1H NMR (400 MHz, DMSO-$d_6$) δ 8.30 (s, 1H), 8.07 (d, $J$ = 2.6 Hz, 1H), 7.57 (d, $J$ = 2.7 Hz, 1H), 4.40 (s, 2H).

5. Preparation of Compound 6
Under the agitation of the ice bath, 2-chloro-3-hydrazinylpyrazine (5.0 g, 0.069 mol) was added into ethyl acetate (20 mL) ultrasound to mix it, and then slowly added into 2,2,2-trifluoroacetic anhydride (14.5 g, 0.138 mol). After the reaction was completed, NaHCO$_3$ solution and ethyl acetate were directly used for extraction, organic phase was collected, and a brown liquid product (compound 6) (4.57 g, 91.4%) was obtained. ESI-MS m/z: [M+H]$^+$ 336.99, 1H NMR (400 MHz, DMSO-$d_6$) δ 8.95 (s, 1H), 7.94 (d, $J$ = 2.0 Hz, 1H), 7.44 (d, $J$ = 2.1 Hz, 1H).
6. Preparation of Compound 7
First put PPA in 60 ~ 70°C, when it is able to flow, and then it under 100°C to join in the flask containing compound 6 (5.0 g, 336.5 mol) mechanical agitation under reflux device. After the reaction was completed, saturated saline water ultrasound was added to dissolve PPA completely, when NaOH solution was added, the reaction solution was adjusted to be alkaline, with ethyl acetate, retained water phase extraction, then concentrated HCl solution adjusted to acidic solution, and then with ethyl acetate extraction, retained organic phase. After the solvent was whirled with a rotavator, dichloromethane was added and the white solids (compound 7) (4.51 g, 90.2%) were obtained. ESI-MS m/z: [M+H]+ 205.02, 1H NMR (400 MHz, DMSO-d6) δ 11.65 (s, 1H), 7.42 (d, J = 5.4 Hz, 1H), 7.01 (d, J = 5.6 Hz, 1H).

7. Preparation of Compound 1
First under the 80°C, the compound 7 (5.0 g, 204.1 mol) is added to the flask, use of DMF as catalyst, with phosphorus oxychloride as solvent, after the temperature switch to 100°C. After 2h of reaction, the reaction fluid is steamed on a rotavator, after mixing with ice water, the NaOH solution was added to make the reaction liquid alkaline, and finally filtered with petroleum ether to obtain a yellowish solid product (compound 1) (4.79 g, 95.75%). ESI-MS m/z: [M+H]+ 223.00, 1H NMR (400 MHz, DMSO-d6) δ 8.75 (s, 1H), 7.99 (s, 1H).

8. Conclusion
In summary, the synthesis of 3, 8-dichloro-[1, 2, 4] triazolo [4, 3-a] pyrazine (1) from 2, 3-dichloropyrazine was optimized by four steps including substitution reaction, acylation reaction, cyclization reaction and chlorination reaction. Optimization by synthesis method, the reaction time is shortened, the temperature is relatively mild, the byproduct is less, and the yield of the target compound 1 is higher. Its structure was confirmed by 1H NMR.

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