Synthesis of 5-nitro-*N*-(4*H*-1, 2, 4-triazol-4-yl) pyridin-2-amine

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Abstract: 5-nitro-*N*-(4*H*-1, 2, 4-triazol-4-yl) pyridin-2-amine, is an important intermediate for the many biologically active intermediates of targeted anticancer drugs. In this work, a rapid and high yield synthetic method for compound 9 was established. Compound 9 was synthesized from 2-bromoacrylate through three steps including addition, cyclization and nucleophilic substitution. The structure of compound 9 was confirmed by ^{1}H NMR and MS spectrum. The total yield of the three steps was high up to 86%.

1. Introduction

Cancer is the leading cause of early death in nearly 100 countries around the world. The global burden of cancer is increasing due to the growth and aging of the world population, and some frequent carcinogenic behaviors such as smoking [1-2]. As reported, 18.1 million cancer cases and 9.6 million cancer deaths occurred in 2018. In gender analysis, lung cancer is the most common cancer among males, and its mortality rate accounts for 18.4% of cancer deaths. The second leading cause of death is breast cancer in female, accounting for 11.6% of cancer death [3-5].

Recently, treatment approaches for cancer include surgery, radiation therapy and medicatio n. In recent years, chemotherapy has been widely used in the treatment of cancer. However, its efficacy is largely limited by tumor resistance and side effects on normal cells and tissue s. Therefore, it is necessary to develop new types of high-efficiency and low-toxicity antitum or drugs. With the deepening research of the mechanisms of drug resistance in cancer cells, molecular cancer therapeutics has great research prospects. Compared with traditional chemot herapy, molecular targeted therapy has the advantages of strong specificity, high efficacy and less damage to normal tissues. Recently, many small molecule inhibitors have been develop ed and in clinical trials [6-10]. For example, 5-nitro-*N*-(4-nitrophenyl)-*N*-(4*H*-1,2,4-triazol-4-yl) pyridin-2-amine(1)[11], 3-(1-((2-((4*H*-1,2,4-triazol-4-yl)amino)pyridin-3-yl)oxy)ethyl)-4-methyl-*N*-(4-methylbenzyl)benzamide(2)[12],4-(4-((6-(dimethylamino)pyridin-3-yl)amino)-5-hydroxy-4*H*-1,2, 4-triazol-3-yl)-6-isopropylbenzene-1,3-diol(3)[13],4-(4-((6-(dimethylamino)pyridin-3-yl)amino)-5-mercapto-4*H*-1,2,4-triazol-3-yl)-6-isopropylbenzene-1,3-diol(4)[13]. It has been observed that m ost of these inhibitors have similar structural of 5-nitro-*N*-(4*H*-1, 2, 4-triazol-4-yl) pyridin-2-a mine (9). The structures of these compounds were shown in Fig.1. Among them, compound 1 has aromatase blocking activity, which can be used to prevent and treat breast cancer, end ometriosis, and prostate hypertrophy. Compound 2 is a ROS1 inhibitor with good in vitro in hibitory activity and selectivity. Compounds 3 and 4 are promising to inhibit Hsp90 and cou ld inhibit the synthesis of many oncoproteins, hormone receptors and transcription factors.

Therefore, the design and synthesis of small molecule inhibitor 5-nitro-*N*-(4*H*-1, 2, 4-triazol-4-yl) pyridin-2-amine (9) derivatives play an important role in the research of targeted anticancer drugs. In this paper, we have discovered an efficient synthetic method for the synthesis of 5-nitro-*N*-(4*H*-1, 2, 4-triazol-4-yl) pyridin-2-amine (9). In the whole process, the target compound 9 was synthesized from reduction reaction, cyclization reaction and nucleophilic reaction, making it more suitable for industrial production [14]. Fig 1. Shows the synthetic route of target compounds.

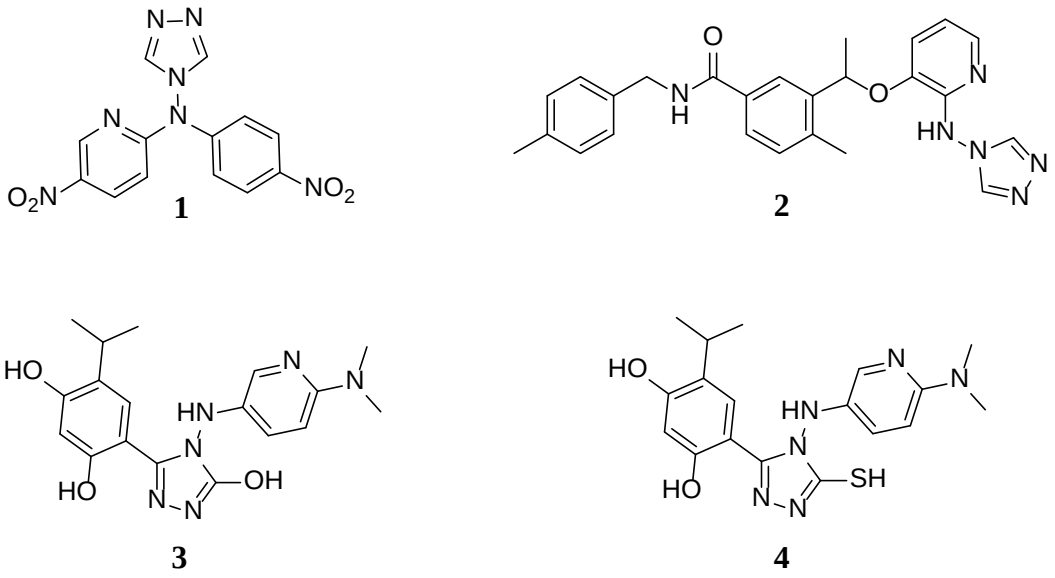

Fig 1. Examples of biologically active molecules bearing the intermediate.

2. Materials and methods

^{1}NMR spectra were performed using Bruker 400 MHz spectrometers (Bruker Bioscience, Billerica, MA, USA) with TMS 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

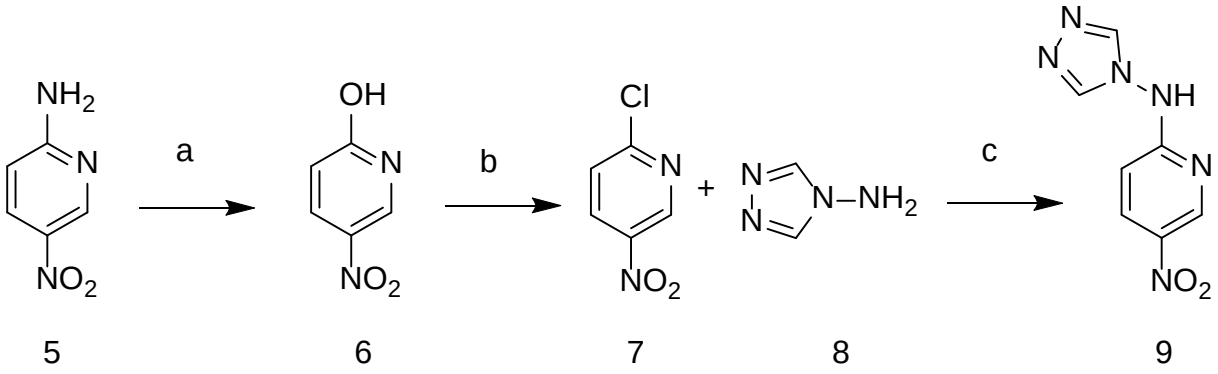

Fig 2. The synthetic route.

Reagents and conditions:(a) $NaNO_2$, HCl, 6 ^{o}C, 40 min; (b) $POCl_3$, ethyl acetate, 140 ^{o}C, 2 h; (c) DIPEA, 1,4-dioxane, acetonitrile, K_2CO_3, 80 ^{o}C, 5 h.

3.1 Preparation for 2-hydroxy-5-nitropyridine (6)

2-Amino-5-nitropyridine (5) (13.9 g, 0.1 mol) was dissolved in 15% hydrochloric acid (55.6 g), then a 20% aqueous solution of $NaNO_2$ (44.85 g) was added dropwise to the mixed solution below 0 ^{o}C. After the addition was completed, the reaction was stirred for 40 minutes at 6 ^{o}C with TLC thin layer technique showed that the starting material had been completely consumed. The solvent was evaporated under reduced pressure. After purification by column chromatography washed with ice water, and filtered, dried to obtain a yellow solid of 2-hydroxy-5-nitropyridine (6), with a yield of 88.02%. MS (ESI): m/z $[M+H]^{+}$ 141.02.

3.2 Preparation for 2-chloro-5-nitropyridine (7)

Compound 6 (5.0 g, 0.04 mol) was dissolved in $POCl_3$ (5.55 g, 0.04 mol) and stirred at 140 ^oC for 2 h. After TLC thin layer technique showed that the starting material had been completely consumed, the solution was washed with ice water (200 mL) and extracted with ethyl acetate (3×100 mL). The combined organic layers were washed with saturated solution of $NaHCO_3$, dried over Na_2SO_4, filtered, and concentrated under reduced pressure to give the title compound 2-chloro-5-nitropyridine (**7**) as yellow solid in 93.5 % yield. MS (ESI): m/z $[M+H]^+$ 158.99.

3.3 Preparation for 5-nitro-N-(4H-1, 2, 4-triazol-4-yl) pyridin-2-amine (9)

A mixture of compound 7 (0.20 g, 1.3 mmol), compound 8 (0.32 g, 3.8 mmol) and K_2CO_3 (0.17 g) was dissolved in 1, 4-dioxane (50 mL). Then *N*, *N*-dimethylformamide (0.5 mL) was added dropwise and refluxed for 5 h at 80 ^oC. The reaction was completed by TLC analysis. The reaction solution was extracted with ethyl acetate. Then the organic layer was concentrated under reduced pressure and recrystallized using petroleum ether and isopropyl alcohol to give a yellow compound 5-nitro-N-(4H-1,2,4-triazol-4-yl)pyridin-2-amine (9) in 86.5% yield. MS (ESI): m/z $[M+H]^+$ 207.06. ^1H NMR (400 MHz, DMSO-d_6) δ 9.55 (s, 1H), 9.37 (d, J = 2.6 Hz, 1H), 8.86 (dd, J = 9.0, 2.6 Hz, 1H), 8.46 (s, 1H), 8.11 (d, J = 9.0 Hz, 1H).

4. Conclusions

In a word, the synthesis of 5-nitro-*N*-(4*H*-1, 2, 4-triazol-4-yl) pyridin-2-amine (9) from 2-Amino-5-nitropyridine was optimized by three steps including cyclization and nucleophilic substitution. With the optimization of reaction conditions and synthesis methods, the reaction route is to prepare a target compound by a safe, green, and highly atomic economy method with higher yield. This work is expected to provide valuable reference for the synthesis of small molecule inhibitors. The structure of compound 5-nitro-*N*-(4*H*-1, 2, 4-triazol-4-yl) pyridin-2-amine (9) was confirmed by MS and ^1H NMR spectrum.

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