Synthesis of 3-substitutedmethylene-2H-thiopyrano[2,3-b]Pyridine-4(3H)-ones and Their Antifungal Activity In Vitro

Yajun Zheng
School of Pharmacy, Pharmaceutical Sciences College of Hebei University
Baoding 071002, Hebei, China
E-mail: hainideyajun@163.com

Zhengyue Ma
School of Pharmacy, Pharmaceutical Sciences College of Hebei University
Baoding 071002, Hebei, China
E-mail: mazhengy@126.com

Xinghua Zhang & Ning Yang
School of Pharmacy, Pharmaceutical Sciences College of Hebei University
Baoding 071002, Hebei, China

Gengliang Yang (Corresponding author)
School of Pharmacy, Pharmaceutical Sciences College of Hebei University
Baoding 071002, Hebei, China
E-mail: ygl@hbu.edu.cn

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Abstract
Six (Z)-3-substitutedmethylene-2H-thiopyrano[2,3-b]pyridin-4(3H)-ones were designed and synthesized. Their structures were confirmed by MS and 1H-NMR and element analysis. Their antifungal activity was tested by microdilution broth susceptibility for eight kinds of fungi, and the results showed that the target compounds exhibited activity against fungi tested to some extent. The compound 5a had the best antifungal effect among of the target compounds.

Keywords: 2H-thiopyrano[2,3-b]pyridin-4(3H)-one, Synthesis, Antifungal activity

1. Introduction
In recent years, invasive fungal infections, especially in those individuals with immunocompromised hosts such as cancer patients and patients with AIDS (N. H. Georgopapadakou, 1996), have continued to increase in incidence. Pyridine derivatives had been reported to possess important biological activities, such as antihypertensive, antitumor, antifungal and so on (Tian Laijin, 2004; Wang Dawei, 2004). Some of 4-oxothiopyrano[2,3-b]pyridine derivatives were recently reported as potential antihypertensive agents (A. D. Settimo, 2000; P. L. Ferrarini, 2000). α,β-unsaturated compounds have also exhibited excellent antitumor, antiinflammatory, antimalaria and other pharmacological effects (T Al Nakibl, 1990; Prithwiraj De, 2010; Bimal K. Banik, 2010; Giovanna Damia, 2009; Peng-Cheng Lv, 2010). At present, the (Z)-3-substitutedmethylene-2H-thiopyrano[2,3-b]pyridine-4(3H)-ones are rarely reported, and their antifungal activity are not reported. On this basis, we design and synthesis of six (Z)-3-substitutedmethylene-2H-thiopyrano[2,3-b]pyridine-4(3H)-ones. Firstly the intermediate of 2H-thiopyrano[2,3-b]pyridin-4(3H)-one was synthesized from the 2-chloronicotinic acid. Secondly, the target compounds were obtained by the reactions of aldehyde with 2H-thiopyrano[2,3-b]pyridin-4(3H)-one in ethanol. The antifungal activity of the target compounds in vitro was measured by consecutive double dilution. The synthetic route was outlined in Figure 1.
2. Experimental

2.1 Chemistry material

2-chloronicotinic acid (chemically pure) were from SHANDONG KEHUI Chemical Co., LTD (SHANDONG, China), and the other reagents were almost from TIANJIN Chemical LLC (TIANJIN, China). 1H-NMR spectra were recorded in CDCl₃ on Bruker Avance DMX 600 using TMS as an internal standard (Bruker, Billerica, MA, USA). Mass spectral data were obtained by LC-MSD Trap XCT G2446A (Agilent Technologies, USA). Melting points were determined SGW X-4 microscopic melting point (Shanghai Precision & Scientific Instrument Co., Ltd, China). Elemental Analysis (C, H, N, S) was realized on Carlo Erba 1106 EA instrument.

2.2 Preparation of 2-mercaptanonic acid

A suspension of 2-chloronicotinic acid 1 (15.7 g, 100 mmoles) and thiocarbamide (13.7 g, 180 mmoles) in 170 mL of water was strong mixing reflux for 4 hours. After cooling, the solid precipitate product was collected and washed with water to give 14.8 g (95% yield) of pure 2.

2.3 Preparation of 2-(2-carboxyethylthio)nicotinic acid

3-chloropropionic acid (11.7 g, 108 mmoles) and sodium iodide in 50 mL of water and sodium hydrogen carbonate (9 g, 108 mmoles) were added to a solution of 2-mercaptopyrindine-3-carboxylic acid (13.9 g, 90 mmoles) in 90 mL of 10% potassium hydroxide aqueous solution. The reaction mixture was stirred at 60°C for 3 hours, cooled and acidified with concentrated hydrochloric acid to pH 3. The solid precipitate product was collected and washed with water to give 18.7 g (92% yield) of pure 3.

2.4 Preparation of 2H-thiopyran[2,3-b]pyridin-4(3H)-one

2.5 Synthesis of (Z)-3-(2-methylpropylidene)-2H-thiopyran[2,3-b]pyridin-4(3H)-one (5a-5f)

A solution of potassium hydroxide (1.3 g, 24 mmoles) and compound 4 (3.3 g, 20 mmoles) in 7 mL of water and 12 mL of ethanol were taken into a 50 mL round-bottomed flask, after which, isobutyraldehyde (2.1 g, 20 mmoles) was added over 10 minutes at room temper ature, and then the mixture was stirred for 3 hours at temperature 25-30°C. After cooling, the solid precipitate product was collected to give 4.5 g of crude 5. Purification was made by filtration on a silica gel chromatographic column, using petroleum ether 60-80°C/ethyl acetate 10:1 as the eluting system. The product recovered from the less mobile fraction gave 3.5 g (25% yield) of pure 5.

2.5.1 (Z)-3-(2-methylpropylidene)-2H-thiopyran[2,3-b]pyridin-4(3H)-one (5a)

Pale yellow viscous liquid, yield 62%; 1H-NMR(600 MHz, CDCl₃): δ 4.02(s, 2 H, H-2), 6.21(d, J=3.04 Hz, 1 H), 6.36-6.34(m, 2 H, H-9), 3.93(s, 2 H, H-2), 6.72(d, J=10.06 Hz, 1 H, H-8), 7.18(dd, J=7.90, 4.63 Hz, 1 H, H-6), 8.40(dd, J=7.90, 1.86 Hz, 1 H, H-5), 8.53(dd, J=4.62, 1.86 Hz, 1 H, H-7); APCI(m/z+H): 244.0; Anal. calcd for C₁₃H₁₃ClNO₂S(%): C, 65.72; H, 5.97; N, 6.39; S, 14.62; Found (%): C, 65.63; H, 5.95; N, 6.40; S, 14.65.

2.5.2 (Z)-3-(furan-2-ylmethylene)-2H-thiopyran[2,3-b]pyridin-4(3H)-one (5b)

Yellow crystals; mp 100-102°C; yield 59%; 1H-NMR(600 MHz, CDCl₃): δ 4.02(s, 2 H, H-2), 6.21(d, J=3.04 Hz, 1 H), 6.36-6.34(m, 2 H, H-9), 3.93(s, 2 H, H-2), 6.72(d, J=1.50 Hz, 1 H, H-2), 7.38(d, J=8.14, 4.50 Hz, 1 H, H-6), 7.64(s, 1 H, H-8), 8.78(dd, J=4.49, 1.82 Hz, 1 H, H-5), 8.81(dd, J=8.18, 1.82 Hz, 1 H, H-7); APCI(m/z+H): 220.0; Anal. calcd for C₁₂H₁₁ClNO₂(%): C, 64.18; H, 3.73; N, 5.76; Found (%): C, 64.21; H, 3.72; N, 5.77; S, 13.15.

2.5.3 (Z)-3-(4-methoxybenzylidene)-2H-thiopyran[2,3-b]pyridin-4(3H)-one (5c)

Yellow crystals; mp 109-110°C; yield 70%; 1H-NMR(600 MHz, CDCl₃): δ 3.94(s, 2 H, H-2), 3.82(s, 3 H, H-11), 6.92-6.89(m, 2 H, H-10), 7.20(t, J=5.78 Hz, 2 H, H-9), 7.50(dd, J=8.25, 4.42 Hz, 2 H, H-6, H-8), 8.79(dd, J=4.49, 1.88 Hz, 1 H, H-5), 8.83(dd, J=8.06, 1.89 Hz, 1 H, H-7); APCI(m/z+H): 484.0; Anal. calcd for C₁₆H₁₃ClNO₂S(%): C, 67.82; H, 4.62; N, 4.94; S, 11.32; Found (%): C, 67.78; H, 4.59; N, 4.95; S, 11.33.

2.5.4 (Z)-3-(4-nitrobenzylidene)-2H-thiopyran[2,3-b]pyridin-4(3H)-one (5d)

Yellow crystals, mp 159-160°C; yield 68%; 1H-NMR(600 MHz, CDCl₃): δ 4.10(s, 2 H, H-2), 7.48(d, J=8.65 Hz, 2 H, H-9), 7.52(dd, J=8.14, 4.50 Hz, 1 H, H-6), 7.74(s, 1 H, H-8), 8.19(d, J=7.69 Hz, 2 H, H-10), 8.79(dd, J=8.15, 1.87
In conclusion, the target compounds had an antifungal effect on most tested fungi. C. neoformans exhibited activity against fungi tested to some extent. And all the target compounds had no activity against C. albicans.

2.6 Antifungal Activity in Vitro

In vitro antifungal activities were measured by means of the minimal inhibitory concentrations (MIC) by consecutive double dilution method. The MIC means the lowest concentration of an antimicrobial agent that prevents visible growth of a microorganism in broth dilution susceptibility test (Marcelo C. Murguía, 2008). The MIC was determined according to the national committee for clinical laboratory standards (NCCLS) recommendation. Eight human opportunistic pathogenic fungi (C. parapsilosis, C. glabrata, C. albicans, C. tropicalis, C. neoformans, C. krusei, A. niger, M. gypseum) were tested. All experiments were performed in comparison with Fluconazole, a known antifungal agent (Odds, F. C, 1986, Hoban, D. J., Zhanel, G. G., Karlowsky, J. A. (1999). In Vitro Susceptibilities of Candida and Cryptococcus neoformans Isolates from Blood Cultures of Neutropenic Patients. Antimicrobial Agents and Chemotherapy, 43: 1463.

In conclusion, the target compounds had an antifungal effect on most tested fungi in vitro. Compound 5a had the best antifungal effect among of the target compounds. Further biological evaluation of the compounds is in progress.
Table 1. Antifungal activity of compounds synthesized in vitro

<table>
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Abbreviations: Cp, C.parapsilosis; Cg, C.glabrata; Ca, C.albicas; Ct, C.tropicalis; Cn, C.neoformans; CK, C.Krusei; An, A.niger; Mg, M.gypseum; Flu, Fluconazole.
Figure 1. Synthesis route of target compounds