

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

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Abstract

Six (Z)-3-substitutedmethylene-2*H*-thiopyrano[2,3-*b*]pyridin-4(3*H*)-ones were designed and synthesized. Their structures were confirmed by MS and ¹H-NMR and element analysis. Their antifungal activity was tested by micro dilution 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: 2*H*-thiopyrano[2,3-*b*]pyridin-4(3*H*)-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-2*H*-thiopyrano[2,3-*b*]pyridine -4(3*H*)-ones are rarely reported, and their antifungal activity are not reported. On this basis, we design and synthesis of six (Z)-3-substitutedmethylene-2*H*-thiopyrano[2,3-*b*]pyridin-4(3*H*)-ones. Firstly the intermediate of 2*H*-thiopyrano[2,3-*b*]pyridin-4(3*H*)-one was synthesized from the 2-chloronicotinic acid. Secondly, the target compounds were obtained by the reactions of aldehyde with 2*H*-thiopyrano[2,3-*b*]pyridin-4(3*H*)-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). $^1\text{H-NMR}$ spectra were recorded in CDCl_3 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-mercaptonicotinic acid

A suspension of 2-chloronicotinic acid 1 (15.7 g, 100 mmol) and thiocarbamide (13.7 g, 180 mmol) 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 mmol) and sodium iodide in 50 mL of water and sodium hydrogen carbonate (9 g, 108 mmol) were added to a solution of 2-mercaptopyridine-3-carboxylic acid (13.9 g, 90 mmol) in 90 mL of 10% potassium hydroxide aqueous solution. The reaction mixture was stirred at 60°C for 4 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-thiopyrano[2,3-b]pyridin-4(3H)-one

A solution of 3 (19.3 g, 85 mmol) and anhydrous sodium acetate (13.9 g, 170 mmol) in 72 mL of acetic anhydride was refluxed at 160°C for 1.5 hours. After cooling, the reaction mixture was diluted with water, basified with 30% ammonium hydroxide solution to pH 8-9, extracted with ethyl acetate. The combined extracts were washed with water, dried and evaporated to give 9.7 g of crude 4. Purification was made by filtration on a silica gel chromatographic column, using petroleum ether $60-80^\circ\text{C}$ /ethyl acetate 10:1 as the eluting system. The product recovered from the less mobile fraction gave 3.5 g (25% yield) of pure 4.

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

A solution of potassium hydroxide (1.3 g, 24 mmol) and compound 4 (3.3 g, 20 mmol) 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 mmol) was added over 10 minutes at room temperature, and then the mixture was stirred for 3 hours at temperature $25-30^\circ\text{C}$. After cooling, the solid precipitate product was collected to give 4.5 g of crude 5a. Purification was made by filtration on a silica gel chromatographic column, using petroleum ether $60-80^\circ\text{C}$ /ethyl acetate 10:1 as the eluting system. The product recovered from the less mobile fraction gave 3.1 g (62% yield) of pure 5a.

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

Pale yellow viscous liquid, yield 62%; $^1\text{H-NMR}$ (600 MHz; CDCl_3) δ : 1.15(d, $J=6.63$ Hz, 6H, H-10), 2.78(sext.t, $J=13.21$, 6.58 Hz, 1 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): 220.0; Anal. calcd for $\text{C}_{12}\text{H}_{13}\text{ClNOS}$ (%): C, 65.72; H, 5.97; N, 6.39; S, 14.62; Found (%): C, 65.83; H, 5.95; N, 6.40; S, 14.65.

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

Yellow crystals; mp $100-102^\circ\text{C}$; yield 59%; $^1\text{H-NMR}$ (600 MHz, CDCl_3) δ : 4.02(s, 2 H, H-2), 6.21(d, $J=3.04$ Hz, 1 H), 6.36-6.34(m, 1 H, H-10), 7.38(d, $J=1.50$ Hz, 1 H), 7.49(dd, $J=8.14$, 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): 244.0; Anal. calcd for $\text{C}_{13}\text{H}_9\text{ClNO}_2\text{S}$ (%): C, 64.18; H, 3.73; N, 5.76; S, 13.18; Found (%): C, 64.21; H, 3.72; N, 5.77; S, 13.15.

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

Yellow crystals; mp $109-110^\circ\text{C}$; yield 70%; $^1\text{H-NMR}$ (600 MHz, CDCl_3) 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 $\text{C}_{16}\text{H}_{13}\text{ClNO}_2\text{S}$ (%): C, 67.82; H, 4.62; N, 4.94; S, 11.32; Found (%): C, 67.80; H, 4.59; N, 4.95; S, 11.33.

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

Yellow crystals, mp $159-160^\circ\text{C}$, yield 68%; $^1\text{H-NMR}$ (600 MHz, CDCl_3) 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

Hz, 1 H, H-5), 8.82(dd, $J=4.50$, 1.86 Hz, 1 H, H-7); APCI(m/z+H): 299.0; Anal. calcd for $C_{15}H_{10}ClN_2O_3S$ (%): C, 60.39; H, 3.38; N, 9.39; S, 10.75; Found (%): C, 60.36; H, 3.37; N, 9.40; S, 10.73.

2.5.5 (Z)-3-(benzo[d][1,3]dioxol-5-ylmethylene)-2H-thiopyrano[2,3-b]pyridin-4(3H)-one (5e)

Yellow crystals; mp 110-112 °C; yield 57%; 1H -NMR(600 MHz, $CDCl_3$) δ : 8.83(dd, $J=8.16$, 1.86 Hz, 1 H, H-7), 8.79(dd, $J=4.48$, 1.86 Hz, 1 H, H-5), 7.54(s, 1 H, H-8), 7.50(dd, $J=8.12$, 4.49 Hz, 1 H, H-6), 5.97(s, $J=5.78$ Hz, 2 H, H-10), 3.91(s, 2 H, H-2), 6.78(td, $J=10.30$, 7.79 Hz, 3 H, H-9, H-11, H-12); APCI(m/z+H): 298.0; Anal. calcd for $C_{16}H_{11}ClNO_3S$ (%): C, 64.63; H, 3.73; N, 4.71; S, 10.78; Found (%): C, 64.61; H, 3.70; N, 4.72; S, 10.81.

2.5.6 (Z)-3-(4-(dimethylamino)benzylidene)-2H-thiopyrano[2,3-b]pyridin-4(3H)-one (5f)

Pale yellow oily; yield 66%; 1H -NMR(600 MHz, $CDCl_3$) δ : 2.94(s, 6 H, H-11), 3.88(s, 2 H, H-2), 6.74(d, $J=8.50$ Hz, 2 H, H-10), 7.13(d, $J=8.57$ Hz, 2 H, H-9), 7.47(dd, $J=8.11$, 4.48 Hz, 1 H, H-6), 7.45(s, 1 H, H-8), 8.81(dd, $J=8.08$, 1.88 Hz, 1 H, H-7), 8.76(dd, $J=4.49$, 1.87 Hz, 1 H, H-5); APCI(m/z+H): 297.1; Anal. calcd for $C_{17}H_{16}ClN_2OS$ (%): C, 68.89; H, 5.44; N, 9.45; S, 10.82; Found (%): C, 68.88; H, 5.48; N, 9.39; S, 10.86.

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. Murgui'a, 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, 1999). The six new compounds were dissolved in dimethyl sulfoxide (DMSO) (1 mL), further progressive dilutions by RPMI 1640 gave there quired concentrations (64, 32, 16, 8, 4, 2, 1, 0.5, 0.25, 0.125 μ g/mL); the fungi were prepared and adjusted to a final concentration of 0.5×10^4 - 2.5×10^4 CFU/mL. MIC values were determined by visual observation after 2-7 d of incubation.

3. Results and discussions

In order to make the reaction proceed easy, we put sodium iodide into the synthetic process of compound 3 on the basis of literature (A. D. Settimo, 2000; P. L. Ferrarini, 2000). The compounds 5a-5f were synthesized by *Knoevenagel* reaction of compounds 4. With active methylene compound 4 and aldehyde condensation in the presence of alkaline catalysts are α,β -unsaturated ketones.

The results of antifungal activities *in vitro* were shown in Table 1. The results showed that the target compounds exhibited activity against fungi tested to some extent. And all the target compounds had no activity against *C.neoformans*. The compound 5a showed a similar level of activity with Fluconazole when against *M.gypseum* and *C.Krusei*, and showed moderate activity against *C.glabrata*.

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.

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Table 1. Antifungal activity of compounds synthesized *in vitro*

Compound	MIC ₁₀₀ /($\mu\text{g}\cdot\text{L}^{-1}$)							
	Cp	Cg	Ca	Ct	Cn	CK	An	Mg
5a	64	32	64	>64	—	64	>64	64
5b	>64	>64	>64	>64	—	>64	>64	>64
5c	>64	>64	>64	>64	—	>64	>64	>64
5d	>64	>64	>64	>64	—	>64	>64	>64
5e	>64	>64	>64	>64	—	>64	>64	>64
5f	>64	>64	>64	>64	—	>64	>64	>64
Flu	4	16	0.5	2	4	64	>64	64

Abbreviations: Cp, *C.parapsilosis*; Cg, *C.glabrata*; Ca, *C.albicans*; Ct, *C.tropicalis*; Cn, *C.neoformans*; CK, *C.Krusei*; An, *A.niger*; Mg, *M.gypseum*; Flu, Fluconazole.

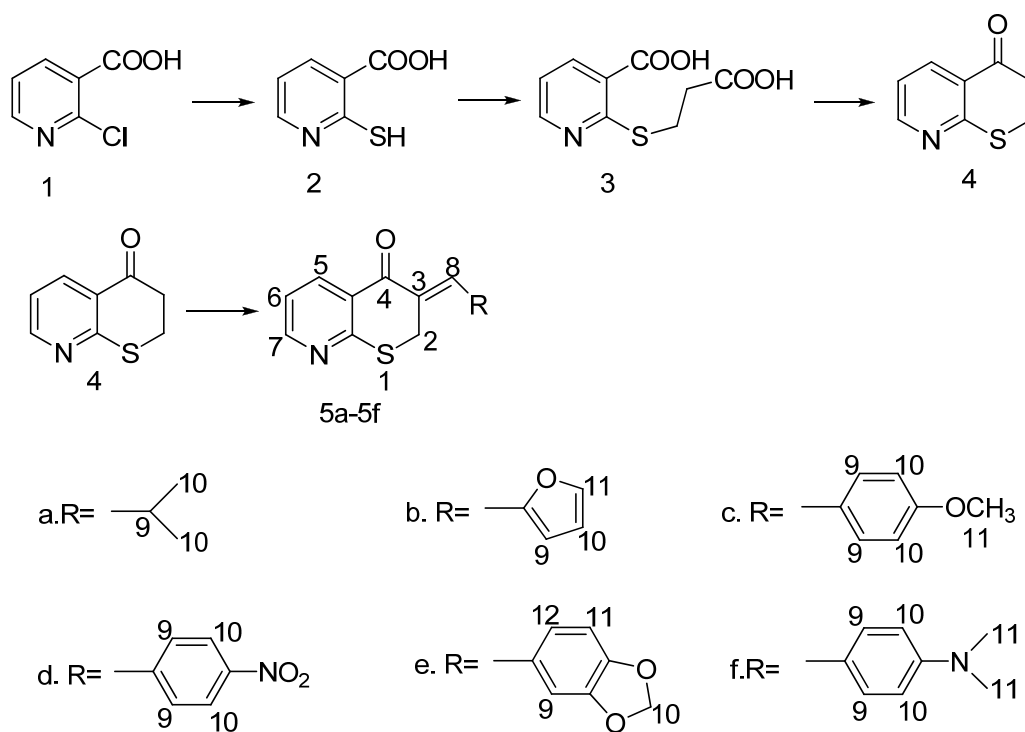


Figure 1. Synthesis route of target compounds