Design, Synthesis and Antifungal Activity of 3-substituedmethylenethiochroman-4-one Derivatives

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Abstract

Four new 3-substituedmethylenethiochroman-4-one derivatives were prepared from (z)-3-(chloromethylene)thiochroman-4-one. They were characterized by analytical and spectral methods. *In vitro* antifungal activities of these synthesized compounds were evaluated against ten species of fungi, and the results showed that (e)-3-chloromethylene compound exhibited a similar good activity against fungi to (z)-3-chloromethylene compound. (Z)-3-substituedaminomethylene compound also exhibited antifungi activity to some extent.

Keywords: Antifungal activity, Thiochromanones, 3-substituedmethylenethiochroman-4-one

1. Introduction

Over the last three decades there has been a dramatic increase in the incidence of fungal infections. Discovery of new drugs for the treatment of systemic mycoses is a major challenge in infectious disease research.

Thiochromanones had been reported to possess important biological activities(Nakazumi, H., 1984). Nakib T A *et al* reported that thiochromanone derivatives had antifungal activities (Nakib, T. A. 1990). 3-Benzylidene (Qi Ping, 2004), 3-Mannich base (Zhu Quanhong, 2000), 3-bromo (QI Ping, 2003), 2,3,3a,4-tetrahydrothiochromeno[4,3-*c*]pyrazole derivatives (Ma Zhengyue, 2008) and 6*H*-thiochromeno[4,3-*b*]quinoline derivatives (Wang Ge, 2010) had been synthesized and reported to have antifungal activities. In our laboratory, (z)-3-halomethylene of thiochromanone derivatives were synthesized and proved with good antifungal activity (Fang Baoling, 2010; Tian Wei, 2010). To discover new 3-substituedmethylene analogues with good antifungal activity, herein, four new compounds from the (z)-3-(chloromethylene)thiochroman-4-one were designed, synthesized and screened for their antifungal activity. The synthetic route was outlined in the Scheme 1.

2. Experimental

2.1 Chemistry

Substituted benzenethiols (chemically pure) were from SHOUERFU LLC (ZHEJIANG, China). All other materials were commercially available and used as received unless otherwise noted. Mass spectral data were obtained by LC-MSD XCT Trap G2446A (Agilent Technologies, USA). Melting points were determined SGW

X-4 microscopic melting point (Shanghai Precision & Scientific Instrument Co., Ltd, China). IR spectra were recorded in potassium bromide on FTIR-8400S (SHIMADZUCO-RPORATION, Kyoto, Japan). ^{1}H NMR spectra were recorded in $CDCl_3$ on Bruker Avance III 600Hz spectrometer. The chemical shifts are reported as parts per million (δ ppm) from $(CH_3)_4Si$ (TMS) as an internal standard. Elemental analysis was performed on a Carlo Erba-1106 instrument and the results were in acceptable range.

2.2 Preparation of compound (z)-3-(chloromethylene)-6-chlorothiochroman-4-one (3)

Sodium methoxide (20.0 mmol), ethyl formate (10.0 mmol) and toluene (50 mL) were mixtured into a round-bottomed flask (250 mL), then a solution of compound 1 (5.0 mmol) in toluene (20 mL) were dropwise added over 20 min in ice bath. The mixture was stirred for 12 h at the temperature < 10 ^{o}C. The organic phase was extracted twice with water (2×20 mL), the combined aqueous phase was adjusted to about 4 with HCl, maintaining the temperature at < 5 ^{o}C, the solid precipitated was filtered, abundantly washed with water, then air dried. The crude product was recrystallized from 95% (v/v) EtOH to afford the compound 2.

compound 2 (5.0 mmol) and acetyl chloride (7.5 mmol) was dissolved in dichloromethane (40 mL) in a sealed tube, and the mixture was stirred at 50 °C for 2 h. After the the solution was extracted with 0.5 mol/L Na_2CO_3 (2×15 mL), the organic layer was was dried over anhydrous $MgSO_4$ and evaporated in vacuo to give the crude product. The crude product was purified by silica-gel column chromatography (dichloromethane: petroleum ether=1:10 (v/v)) to afford the compound 3 (Tian Wei, 2010; Fang Baoling, 2010), compound 2: yield, 83%; compound 3: yield, 74%.

2.2 Preparation of compound (e)-3-(chloromethylene)-6-chlorothiochroman-4-one (4)

compound 3 (5.0 mmol) was dissolved in methanol (60 mL) in a round bottomed flask (150 mL)irradiating 20 h under 60 W UV lamp, solvent was evaporated in vacuo to give the crude product. The crude product was purified by silica-gel column chromatography (dichloromethane: petroleum ether=1:10(v/v).) to afford the pure compound 4.

2.2.1 (E)-6-Chloro-3-(chloromethylene)thiochroman-4-one (4)

Yellow solid. Yield: 30%; mp: 91-93 °C. UV-vis (MeOH) λ_{max}: 250 nm; ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm: 8.19 (d, J = 1.68 Hz, 1H, Ar-H), 7.39 (dd, J = 8.35, 1.76 Hz, 1H, Ar-H), 7.25 (d, J = 8.43 Hz, 1H, Ar-H), 6.76 (s, 1H, C=CH-),3.87 (s, 2H, SCH_2). IR (KBr): 1670 (C=O), 1593 (C=C) cm^{-1}. MS (APCI): *m/z* 244.9 $[M+H]^+$, 246.9 $[M+2+H]^+$. Anal. Calcd for $C_{10}H_6Cl_2OS$: C, 49.00; H, 2.47; S, 13.08. Found: C, 48.96; H, 2.42; S, 13.12.

2.3 Preparation of compound (Z)-6-Chloro-3-((dimethylamino)methylene)thiochroman-4-one (5)

$NH(CH_3)_2$·HCl (6.0 mmol) and $N(CH_2CH_3)_3$ (15.0 mmol) were dissolved in CH_2Cl_2 (25 mL) and cooled to 0 °C, then a solution of compound 3 (5.0 mmol) in CH_2Cl_2 (5 mL) were added, dropwise, and the mixture was stirred at 0 °C for 2.5 h. After the solution was extracted with water (2×20 mL), the organic layer was dried over anhydrous $MgSO_4$ and evaporated in vacuo to give the crude product. The crude product was recrystallized from 95% (v/v) EtOH to afford the compound **5.**

2.3.1 (Z)-6-Chloro-3-((dimethylamino)methylene)thiochroman-4-one (6)

Yellow solid. Yield: 87%; mp: 123-125 °C. UV-vis (MeOH) λ_{max}: 248 nm; ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm: 8.06 (d, J = 2.35 Hz, 1H, Ar-H), 7.61 (s, 1H, C=CH-), 7.25 (dd, J = 8.33, 2.39 Hz, 1H, Ar-H), 7.19 (d, J = 8.34 Hz, 1H, Ar-H), 4.01 (s, 2H, SCH_2), 3.17 (s, 6H, $C(CH_3)_2$). IR (KBr): 1637 (C=O), 1579 (C=C) cm^{-1}. MS (APCI): *m/z* 253.9 $[M+H]^+$, 255.9 $[M+2+H]^+$. Anal. Calcd for $C_{12}H_{12}ClNOS$: C, 56.80; H, 4.77; N, 5.52; S, 12.64. Found: C, 56.83; H, 4.80; N, 5.47; S, 12.60.

2.4 Preparation of compound 3-(phenylthiomethylene)thiochroman-4-one (6a-6b)

Substituted benzenethiols (6.0 mmol) and NaH (12.5 mmol) were added in dry THF (25 mL) and cooled to 0 °C, then a solution of compound 3 (5.0 mmol) in THF (8 mL) were added, dropwise. After stirring 2 h, added crushed ice slowly to the mixture and removed excess NaH, then added water (60 mL), the solid precipitated was filtered, abundantly washed with water, then air dried. The crude product was purified by silica-gel column chromatography (dichloromethane: petroleum ether = 1:50 (v/v)) to afford the compound 6a and 6b.

2.4.1 (Z)-6-Chloro-3-(p-tolylthiomethylene)thiochroman-4-one (6a)

Green solid. Yield: 34%; mp: 111-113 °C. UV-vis (MeOH) λ_{max}: 254 nm; ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm: 8.07 (d, J = 2.41 Hz, 1H, Ar-H), 7.88 (s, 1H, C=CH-), 7.39 (d, J = 8.06 Hz, 2H, Ar-H), 7.33 (dd, J = 8.43, 2.38 Hz, 1H, Ar-H), 7.25 (d, J = 10.07 Hz, 1H, Ar-H), 7.21 (d, J = 7.94 Hz, 2H, Ar-H), 3.93 (s, 2H, SCH_2), 2.38 (s, 3H, CH_3). IR (KBr): 2923, 2852 (CH_3), 1683 (C=O), 1635 (C=C) cm^{-1}. MS (APCI): *m/z* 333.0 $[M+H]^+$, 335.9

$[M+2+H]^+$. Anal. Calcd for $C_{17}H_{13}ClOS_2$: C, 61.34; H, 3.94; S, 19.27. Found: C, 61.37; H, 3.90; S, 19.31.

2.3.2 (E)-6-Chloro-3-(p-tolylthiomethylene)thiochroman-4-one (6b)

Green solid. Yield: 30%; mp: 104-106 °C. UV-vis (MeOH) λ_{max}: 254 nm; ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm: 8.12 (d, J = 2.41 Hz, 1H, Ar-H), 7.43 (d, J = 8.09 Hz, 2H, Ar-H), 7.35-7.31 (m, 2H, Ar-H), 7.25 (d, J = 8.39 Hz, 1H, C=CH-), 7.21 (d, J = 7.95 Hz, 2H, Ar-H), 3.87 (d, J = 0.75 Hz, 2H, SCH_2), 2.38 (s, 3H, CH_3). IR (KBr): 2921, 1456, 1394(CH_3), 1683 (C=O), 1635 (C=C) cm^{-1}. MS (APCI): m/z 333.0 $[M+H]^+$, 335.9 $[M+2+H]^+$. Anal. Calcd for $C_{17}H_{13}ClOS_2$: C, 61.34; H, 3.94; S, 19.27. Found: C, 61.30; H, 3.90; S, 19.20.

2.5Antifungal Activity in Vitro

In vitro antifungal activities were determined by double dilution method, the Minimum inhibitory concentration (MIC) were determined in accordance with the methods of the National Committee for Clinical Laboratory Standards (Marcelo C. Murguı´a, 2008). *C. parapsilosis*, *C. glabrata*, *C. albicas*, *C. tropicalis*, *C. neoformans*, *C. Krusei*, *E. floccosum*, *M. gypseum*, *A. niger*, *S. schenekn* were used as tested fungi for this study. Fluconazole was used as the reference drugs for positive control. The tested compounds were dissolved in DMSO(1 mL), then the required concentrati- ons (128, 64, 32, 16, 8, 4, 2, 1, 0.5, 0.25, 0.125 μg/mL) were obtained by two fold serial dilution. The fungi were incubated and adjusted to a final concentration of 0.5×10^4-2.5×10^4 CFU/mL. MIC_{100} values were determined by visual observation after 2-7 d of incubation.

3. Results and discussion

3.1 Structure elucidation

The structures of the target compounds synthesized were established by mass spectroscopy, elemental analysis, ^{1}H-NMR spectral data and NOE spectral data. The configuration of compounds 3 and 4 were assigned by their ^{1}H NMR spectra and ^{1}H-^{1}H NOE spectra. For compound 4, NOE correlations were observed from H of -C=CHCl to H of -SCH_2-, but there was no correlation from H of -C=CHCl to H of -SCH_2- on compound 3, as shown in Figure 1. The NOE spectra confirmed the compound 3 was *Z*-configuration and the compound 4 was *E*-configuration (see Figure 2).

During the synthesis of compound 5, only one product was obtained in the reaction. In order to determine its structure, NOE spectra was also studied. The ^{1}H NMR spectrum of compound **5** revealed three singlet signals at δ 3.17, 4.01 and 7.61 characteristic for H of –$N(CH_3)_2$, H of -SCH_2- and H of -C=CHCl, respectively. NOE correlations were observed from H of -C=CHCl to H of -SCH_2-, but there was no correlation from H of -C=CHCl to H of -SCH_2-, as shown in Figure 1. These NOE's confirmed the absolute *E*-configuration of structure 5 (see Figure 2).

During the synthesis of compound 6, there was no selectivity to the compound 6a and 6b, they were generated at the same time. Their structure were also confirmed by ^{1}H NMR spectra and NOE correlations. NOE correlations were observed from H of -C=CHCl to H of -SCH_2- on compound 5b, and there was no correlation from H of -C=CHCl to H of -SCH_2- of compound 5a, as shown in Figure 1. The NOE spectra of compound 5 were also shown in Figure 2.

3.2 Antifungal Activity in vitro

The results of antifungal activities *in vitro* were shown in Table 1. In order to discover new active compound, structure optimization of compound 3 were operated. Compound 4 as *E*-isomers of 3 was prepared, but the result of antifungal activity indicated that there was no apparent difference between 3 and 4. When chloromethylene was replaced with phenylthiomethylene and dimethylaminomethylene respectively, compound 5, 6a, and 6b were synthesized. The tested result showed that dimethylaminomethylene derivative 5 had antigungal activity to some extent. Compound 5 had the best activity when against *C.neoformans*, its MIC was a similar to Fluconazole. However, phenylthiomethylene derivatives 6a and 6b had no antifungal activity against most of the tested fungi. 6a and 6b had the weak bioactive only against *C.neoformans* and *C.Krusei.*

In conclusion, 3-chloromethylene derivatives had good antifungal activity and other substitued 3-methylene derivatives either had lower activity or no acivity. The result should encourage us to design and synthesize more potent antifungal agents. Further biological evaluation of the compounds is in progress.

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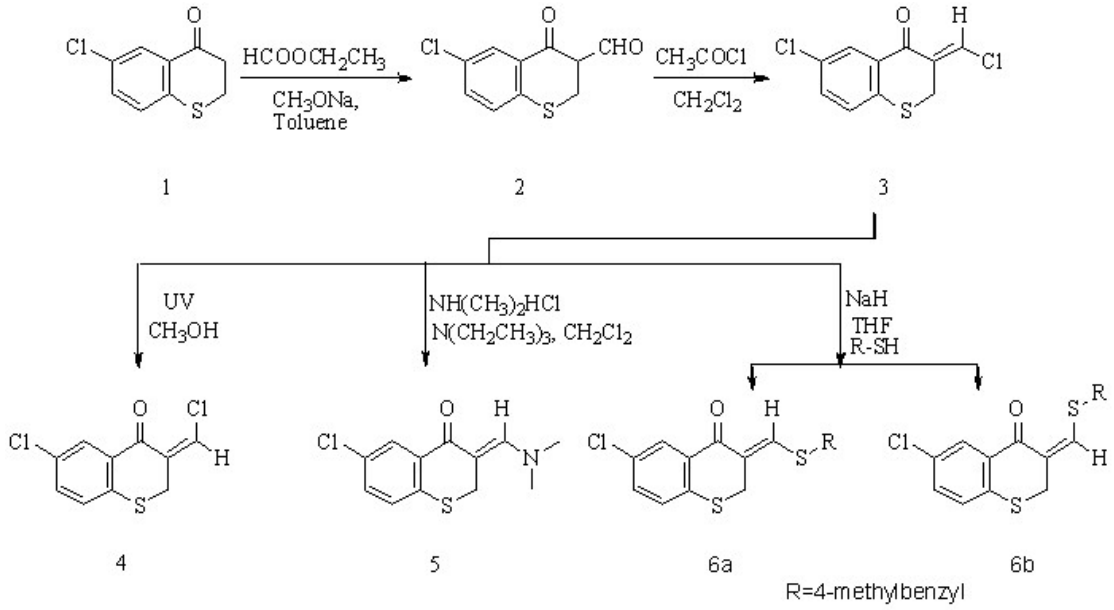
Table 1. Antifungal activity of compounds synthesized *in vitro*

Compound	MIC (μg/mL)									
	Cp	Cg	Ca	Ct	Cn	CK	Ef	Mg	An	Ss
3	2	16	4	4	4	32	2	16	>64	4
4	2	32	4	8	4	16	4	16	>64	4
5	32	>64	32	64	4	64	32	64	32	64
6a	>64	>64	>64	>64	4	32	>64	>64	>64	>64
6b	>64	64	>64	>64	8	64	>64	>64	>64	>64
Flu	4	16	0.5	2	4	64	>64	64	>64	>64

Abbreviations: Cp, *C.parapsilosis*; Cg, *C.glabrata*; Ca, *C.albicas*; Ct, *C.tropicalis*; Cn, *C.neoformans*; CK, *C.Krusei*; Ef, *E.floccosum*; Mg, *M.gypseum*; An, *A.niger*; Ss, *S.schenekn*; Flu, Fluconazole.



Scheme 1. The synthesis route of target compound

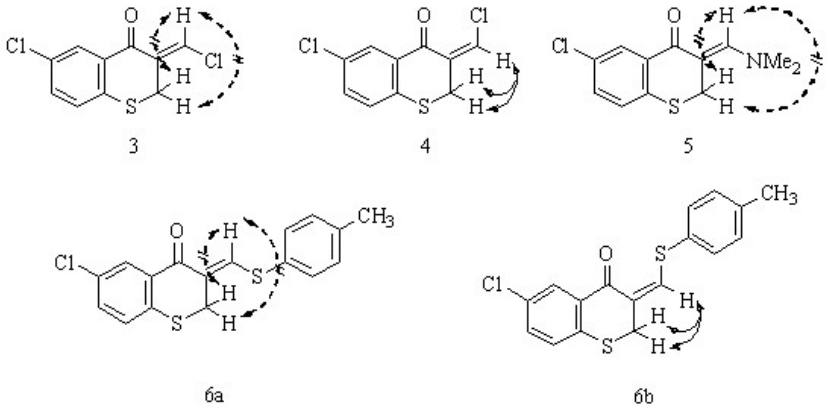
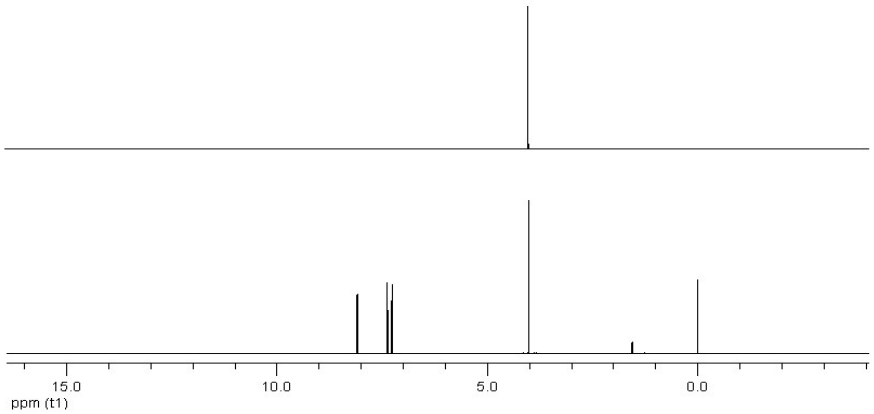
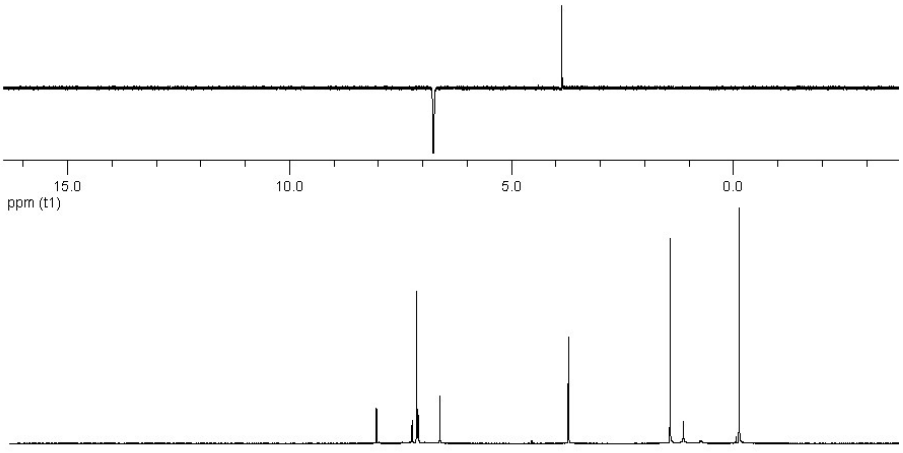


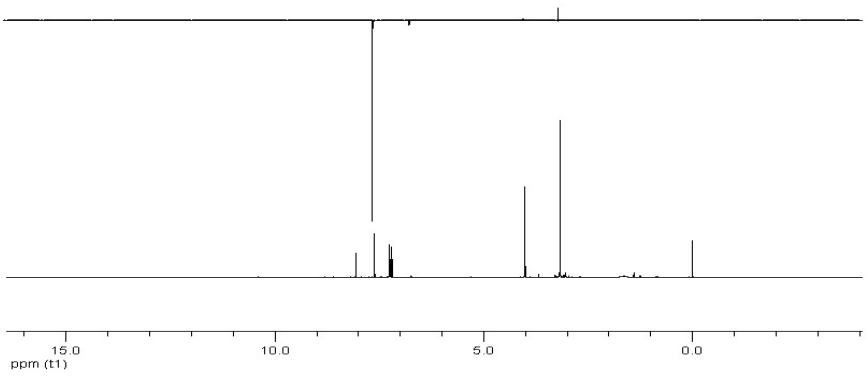
Figure 1. NOE correlations are shown by arrows



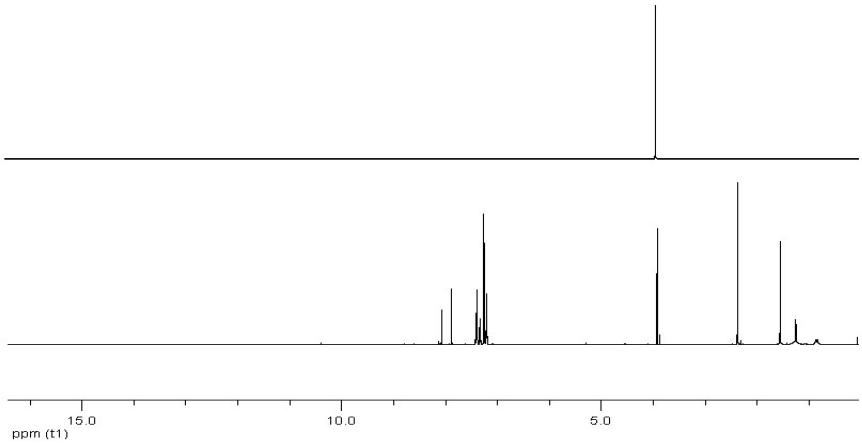
compound 3.



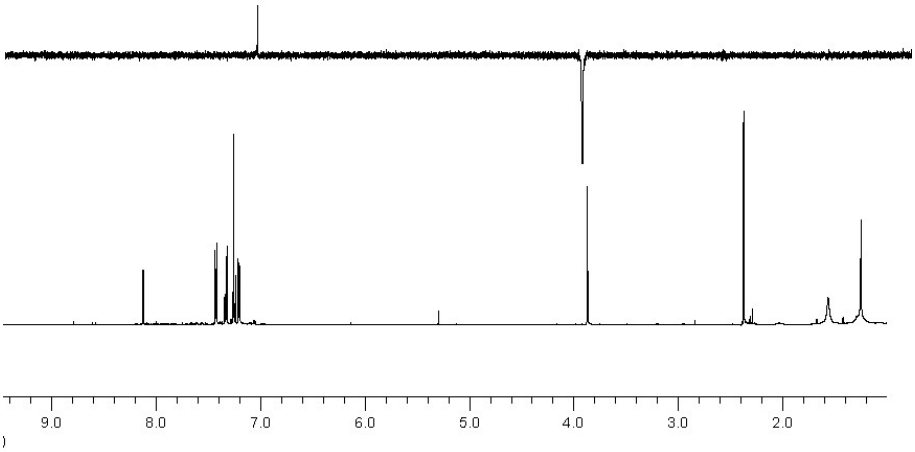
compound 4.



compound 5.



compound 6a.



compound 6b.

Figure 2. ^{1}H NMR spectral and NOE spectral of 3a, 4, 5 and 6