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Synthesis and Antifungal Activity of Some Thiazole Derivatives

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A series of some thiazole derivatives were designed and synthesized. The structure of newly synthesized compounds was characterized by HRMS, ^1H NMR and ^{13}C NMR. The synthesized compounds were evaluated against ten species of fungi *in vitro* by agar cup plate and micro-titration methods, respectively. The results of antifungal screening reveal that among all the compounds screened three compounds showed moderate antifungal activity. The MIC value of **3** against two fungal strains *C. neoformans* and *C. albicans* is $8\ \mu\text{g mL}^{-1}$ respectively. The MIC value of **3i** against two fungal strains *C. neoformans* and *T. mentagrophytes* is $8\ \mu\text{g mL}^{-1}$ and $16\ \mu\text{g mL}^{-1}$, respectively and **3a** against *T. mentagrophytes* is $16\ \mu\text{g mL}^{-1}$, the MIC of others are all beyond $32\ \mu\text{g mL}^{-1}$.

Key Words: Thiochromanone, Schiff base, Thiazole, Antifungal activity.

INTRODUCTION

Schiff bases are well known for their pharmacological properties as antibacterial, antifungal, anticancer and antiviral agents¹. Similarly, thiazole derivatives possess activities such as antibacterial², antifungal³, antiinflammatory⁴, anti-hypertensive⁵, anti-HIV⁶, antitumor⁷ and antifilarial⁸. The potential possibility of hydrazothiazole derivatives for better therapeutic results has attracted extensive interest in last decade. Thiochroman-4-ones are a class of heterocyclic compounds with sulfur atom and possessed a wide range of physiological activities. Thiochromanones had been reported to possess important biological activities⁹. Nakib *et al.* reported that thiochromanone derivatives had antifungal activities¹⁰. Since the thiazole moiety and thiochromanones seems to be a possible pharmacophore in various pharmacologically active agents, we decided to synthesize compounds with these functionality coupled with Schiff base as possible antimicrobial agents which could furnish better therapeutic results. In spite of an enormous number of reports on the utility of these compounds in synthesis of heterocycles, to the best of our knowledge, this subject has never been surveyed¹¹. In this paper, ten thiazole derivatives were designed and synthesized to search for more potential antifungal agents¹². Target compounds are based on different substituents thiochroman-4-ones bromination acetophenone and amino thiourea as raw materials synthesis¹³.

EXPERIMENTAL

^1H NMR and ^{13}C NMR was assayed using Bruker AVIII-600 MHz NMR spectrometer, TMS used as internal standard

and $\text{DMSO-}d_6$ as solvent; HRMS spectra were determined on a Bruker apex ultra 7.0 T Fourier transform mass spectrometer. Melting point of compounds was determined by shenguang SGW X-4 micro melting point detector in open capillaries and is uncorrected. (Shanghai Precision and Scientific Instrument Co. Ltd., China); microwave generating device was MC-3 microwave reactor (Shanghai Jiesi Bio Technology Co. Ltd., China); substituted thiophenol was chemically pure and other reagents were of analytical grade. Control drug amphotericin B was purchased from North China Pharmaceutical Factory and fluconazole from Shijiazhuang Pharmaceutical Factory.

Synthetic method of substituted 4-chromanones 2a-2j: Substituted phenol or thiophenol (0.1 mol) and β -chloropropionic acid (0.12 mol) were placed in 250 mL conical flask and KOH (0.24 mol) was added. The mixture was completely agitated till uniformity and irradiated for 15 min (thiophenol for 10 min) in microwave oven. After the reaction system was cooled to room temperature, the solution was adjusted by concentrated hydrochloric acid to $\text{pH} = 1$. A large number of white precipitate occurred, the resultant solution was filtrated. The obtained filter cake was rinsed with a mass of water and then recrystallized by ethanol/water. After drying, white solid compound **1** was obtained. The compound was dissolved in four times the volume concentrated sulfuric acid, kept at room temperature for 12 h and then placed in the ice water bath for dissociation. Solid precipitation substituted thiochromanone **2** was obtained (**Scheme-I**).

Synthetic method of thiazoles derivatives (3a-3j): Substituted thiochromanone (2 mmol), brominatedacetophenone (2 mmol), thiosemicarbazide (2 mmol) and 10 mL ethanol were mixtured in a 50 mL round-bottomed flask. The mixture was then refluxed for 7-20 h with stirring, the completion of the reaction mixture monitored by TLC. After completion of the reaction, a solid was obtained. The crude product was collected by filtration through Buchner funnel filtrated, washed with a mass of water, dried and recrystallized from ethanol/water, target product **3** was obtained. The authenticity of the products (**3a-3j**) was established by their ^1H NMR, ^{13}C NMR, HRMS data. The approach leading to the compound **3** is illustrated in in **Scheme-I**.

Scheme-I: Synthesis of the target compounds

2-[2-(6-Chlorothiochroman-4-ylidene)hydrazinyl]-5-phenylthiazole (3a): ^1H NMR (600 MHz, DMSO) δ 11.24 (s, 1H, NH), 8.01 (s, 1H, Ar-H), 7.87 (d, J = 7.3 Hz, 2H, Ar-H), 7.42 (t, J = 7.7 Hz, 2H, Ar-H), 7.37 (s, 1H, thiazoleH), 7.35-7.25 (m, 3H, Ar-H), 3.12-3.10 (m, 2H, $-\text{CH}_2-\text{S}$), 3.05-3.03 (m, 2H, $-\text{CH}_2-$). ^{13}C NMR (151 MHz, DMSO) δ 170.01, 143.35, 134.78, 134.58, 133.56, 130.38, 130.35, 129.12, 128.68, 128.14, 126.04, 125.42, 104.99, 28.23, 25.49. HRMS (ESI $^+$) [M+H] $^+$: calcd. for $\text{C}_{18}\text{H}_{15}\text{N}_3\text{S}_2\text{F}$: 372.0396; found: 372.0387.

2-[2-(6-Methylthiochroman-4-ylidene)hydrazinyl]-5-phenylthiazole (3b): ^1H NMR (600 MHz, DMSO) δ 10.07 (s, 1H, NH), 7.88 (d, J = 7.5 Hz, 3H, Ar-H), 7.43 (t, J = 7.7 Hz, 2H, Ar-H), 7.37 (s, 1H, thiazoleH), 7.32 (t, J = 7.3 Hz, 1H, Ar-H), 7.16 (d, J = 7.9 Hz, 1H, Ar-H), 7.08 (dd, J = 7.9, 1.1 Hz, 1H, Ar-H), 3.05 (s, 4H, $-\text{CH}_2-\text{CH}_2-\text{S}$), 2.30 (d, J = 13.4 Hz, 3H, $-\text{CH}_3$). ^{13}C NMR (151 MHz, DMSO) δ 170.24, 145.32, 135.00, 132.47, 131.72, 130.12, 129.12, 128.51, 128.14, 126.67, 126.05, 104.85, 28.98, 25.77, 21.35. HRMS (ESI $^+$) [M+H] $^+$: calcd. for $\text{C}_{19}\text{H}_{18}\text{N}_3\text{S}_2$ 352.0942; Found: 352.0935.

2-[2-(8-Methylthiochroman-4-ylidene)hydrazinyl]-5-phenylthiazole (3c): ^1H NMR (600 MHz, DMSO) δ 11.30 (s, 1H, NH), 7.94 (d, J = 7.9 Hz, 1H, Ar-H), 7.87 (d, J = 7.7 Hz, 2H, Ar-H), 7.42 (t, J = 7.5 Hz, 2H, Ar-H), 7.35 (s, 1H, thiazoleH), 7.31 (t, J = 7.2 Hz, 1H, Ar-H), 7.19 (d, J = 7.2 Hz, 1H, Ar-H), 7.11 (t, J = 7.6 Hz, 1H, Ar-H), 3.07-3.06 (m, 2H, $-\text{CH}_2-\text{S}$), 3.05-3.04 (m, 2H, $-\text{CH}_2-$), 2.25 (s, 3H, $-\text{CH}_3$). ^{13}C NMR (151 MHz, DMSO) δ 170.26, 135.62, 135.49, 131.90, 130.18, 129.11, 128.10, 126.04, 124.94, 124.16, 104.76, 28.46, 25.30, 20.30. HRMS (ESI $^+$) [M+H] $^+$: calcd. for $\text{C}_{19}\text{H}_{18}\text{N}_3\text{S}_2$ 352.0942; found: 352.0934.

2-[2-(8-Fluorothiochroman-4-ylidene)hydrazinyl]-5-phenylthiazole (3d): ^1H NMR (600 MHz, DMSO) δ 11.46 (s, 1H, NH), 7.93 (dd, J = 7.9, 1.1 Hz, 1H, Ar-H), 7.89-7.86 (m, 2H, Ar-H), 7.42 (t, J = 7.7 Hz, 2H, Ar-H), 7.37 (s, 1H, thiazoleH), 7.32 (t, J = 7.3 Hz, 1H, Ar-H), 7.22 (m, 2H, Ar-H), 3.14-3.12 (m, 2H, $-\text{CH}_2-\text{S}$), 3.07-3.05 (m, 2H, $-\text{CH}_2-$). ^{13}C NMR (151 MHz, DMSO) δ 170.09, 157.90, 134.89, 133.86, 129.12, 128.10, 126.08, 126.02, 123.57, 123.44, 122.00, 114.98, 114.84, 104.90, 27.87, 24.62. HRMS (ESI $^+$) [M+H] $^+$: calcd. for $\text{C}_{18}\text{H}_{15}\text{N}_3\text{S}_2\text{F}$ 356.0691; Found: 356.0682.

2-[2-(8-Chlorothiochroman-4-ylidene)hydrazinyl]-5-phenylthiazole (3e): ^1H NMR (600 MHz, DMSO) δ 11.41 (s, 1H, NH), 8.09-8.04 (m, 1H, Ar-H), 7.88 (d, J = 7.3 Hz, 2H, Ar-H), 7.49-7.39 (m, 3H, Ar-H), 7.37 (s, 1H, thiazoleH), 7.32 (t, J = 7.3 Hz, 1H, Ar-H), 7.22 (t, J = 7.9 Hz, 1H, Ar-H), 3.15-3.13 (m, 2H, $-\text{CH}_2-\text{S}$), 3.06-3.04 (m, 2H, $-\text{CH}_2-$). ^{13}C NMR (151 MHz, DMSO) δ 169.58, 134.54, 133.46, 131.01, 128.88, 128.62, 128.31, 127.62, 125.56, 125.53, 124.41, 104.39, 27.12, 24.77. HRMS (ESI $^+$) [M+H] $^+$: calcd. for $\text{C}_{18}\text{H}_{15}\text{N}_3\text{ClS}_2$: 372.0396; Found: 372.0388.

2-[2-(6,7-Dichlorothiochroman-4-ylidene)hydrazinyl]-5-phenylthiazole (3f): ^1H NMR (600 MHz, DMSO) δ 11.54 (s, 1H, NH), 8.10 (s, 1H, Ar-H), 7.86 (d, J = 7.4 Hz, 2H, Ar-H), 7.55 (d, J = 2.1 Hz, 1H, Ar-H), 7.40 (dt, J = 9.4, 4.8 Hz, 2H, Ar-H), 7.35 (s, 1H, thiazoleH), 7.31 (t, J = 7.3 Hz, 1H, Ar-H), 3.13-3.10 (m, 2H, $-\text{CH}_2-\text{S}$), 3.03-3.02 (m, 2H, $-\text{CH}_2-$). ^{13}C NMR (151 MHz, DMSO) δ 169.40, 135.75, 133.22, 132.04, 131.76, 131.59, 130.75, 129.27, 129.18, 129.08, 128.60, 128.27, 127.85, 127.78, 127.62, 126.61, 125.52, 27.33, 24.97. HRMS (ESI $^+$) [M+H] $^+$: calcd. for $\text{C}_{18}\text{H}_{14}\text{N}_3\text{Cl}_2\text{S}_2$: 406.0006; Found : 405.9996.

2-[2-(6-Fluorothiochroman-4-ylidene)hydrazinyl]-5-phenylthiazole (3g): ^1H NMR (600 MHz, DMSO) δ 11.48 (s, 1H, NH), δ 7.88 (d, J = 7.6 Hz, 2H, Ar-H), 7.75 (dd, J = 10.7, 2.8 Hz, 1H, Ar-H), 7.42 (t, J = 7.7 Hz, 2H, Ar-H), 7.38 (s, 1H, thiazoleH), 7.34-7.28 (m, 2H, Ar-H), 7.14 (td, J = 8.4, 2.9 Hz, 1H, Ar-H), 3.09 (t, J = 6.1 Hz, 2H, $-\text{CH}_2-\text{S}$), 3.06-3.01 (m, 2H, $-\text{CH}_2-$). ^{13}C NMR (151 MHz, DMSO) δ 169.57, 160.87, 159.27, 133.34, 130.72, 130.04, 129.99, 128.59, 127.55, 125.51, 116.10, 115.95, 111.59, 111.43, 27.87, 25.19. HRMS (ESI $^+$) [M+K] $^+$: calcd. for $\text{C}_{18}\text{H}_{14}\text{N}_3\text{S}_2\text{FK}$: 394.0250; found: 394.3172.

2-[2-(7-fluoro-6-methylthiochroman-4-ylidene)hydrazinyl]-5-phenylthiazole (3h): ^1H NMR (600 MHz, DMSO) δ 11.41 (s, 1H, NH), 7.87 (d, J = 7.5 Hz, 2H, Ar-H), 7.69 (d, J = 11.4 Hz, 1H, Ar-H), 7.42 (t, J = 7.7 Hz, 2H, Ar-H), 7.36 (s, 1H, thiazoleH), 7.31 (t, J = 7.3 Hz, 1H, Ar-H), 7.20 (d, J = 7.5 Hz, 1H, Ar-H), 3.07 (t, J = 5.8 Hz, 2H, $-\text{CH}_2-\text{S}$), 3.01 (t, J = 5.8 Hz, 2H, $-\text{CH}_2-$), 2.21 (s, 3H, $-\text{CH}_3$). ^{13}C NMR (151 MHz, DMSO) δ 170.07, 160.05, 158.45, 131.19, 130.87, 129.11, 128.79, 128.36, 128.13, 126.06, 111.77, 104.87, 28.33, 25.68, 14.37. HRMS (ESI $^+$) [M+H] $^+$: calcd for $\text{C}_{19}\text{H}_{16}\text{N}_3\text{S}_2\text{F}$: 370.0848; found: 370.0836.

2-[2-(6-Fluorochroman-4-ylidene)hydrazinyl]-5-phenylthiazole (3i): ^1H NMR (600 MHz, DMSO) δ 10.08 (s, 1H, NH), 8.00 (d, J = 2.1 Hz, 1H, Ar-H), 7.87 (d, J = 7.3 Hz, 2H, Ar-H), 7.44-7.39 (m, 3H, Ar-H), 7.34 (s, 1H, thiazoleH), 7.32 (t, J = 7.3 Hz, 1H, Ar-H), 6.90 (d, J = 8.7 Hz, 1H, Ar-H),

4.30-4.28 (m, 2H, -CH₂-O), 2.93 - 2.915 (m, 2H, -CH₂). ¹³C NMR (151 MHz, DMSO) δ 169.36, 155.42, 132.72, 128.62, 128.30, 127.86, 127.67, 125.94, 125.58, 122.58, 119.92, 113.05, 104.24, 64.68, 25.26. MS(ESI+) [M+H]⁺: calcd for C₁₈H₁₅N₃OSF: 340.0920; Found : 340.0911.

2-[2-(6-Bromochroman-4-ylidene)hydrazinyl]-5-phenylthiazole (3j): ¹H NMR (600 MHz, DMSO) δ 11.54 (s, 1H, NH), 7.87 (d, *J* = 7.5 Hz, 2H, Ar-H), 7.59 (dd, *J* = 9.5, 2.8 Hz, 1H, Ar-H), 7.42 (t, *J* = 7.7 Hz, 2H, Ar-H), 7.36 (s, 1H, thiazoleH), 7.32 (t, *J* = 7.3 Hz, 1H, Ar-H), 7.13 (td, *J* = 8.5, 3.2 Hz, 1H, Ar-H), 6.96 (dd, *J* = 9.0, 4.7 Hz, 1H, Ar-H), 4.27 (m, 2H, -CH₂-O), 2.91 (m, 2H, -CH₂). ¹³C NMR (151 MHz, DMSO) δ 169.42, 157.51, 155.95, 152.69, 128.61, 128.30, 127.62, 125.56, 121.64, 119.20, 117.50, 108.99, 108.83, 104.21, 64.73, 25.39. MS(ESI+) [M+H]⁺: calcd. for C₁₈H₁₅N₃OSBr: 400.0119 ; Found :400.0110.

Antifungal activity: Ten prepared compounds were screened for their *in vitro* antifungal activity¹⁴ against *C. neoformans*, *C. albicans*, *C. tropicalis*, *C. parapsilosis*, *A. niger*, *M. gypseum*, *T. rubrum*, *T. mentagrophytes*, *A. fumigatus* and *C. krusei*. The *in vitro* antibacterial activities of thiazole derivatives (**3a-3j**) were compared with amphotericin B and fluconazole¹⁵ from known agar double dilution method (plate method). The minimal inhibitory concentration (MIC) was the lowest concentration of an antimicrobial agent that prevents visible growth of a microorganism in agar medium susceptibility test. The tested compounds (**3a-3j**) along with amphotericin B and fluconazole were prepared in DMSO, diluted by sterile distilled water and added to sterile 1 % glucose peptone medium. The sample was serially diluted (128.0, 64.0, 32.0, 16.0, 8.00 and 4.00 μg mL⁻¹) and added to RPMI1640 HmediumH, after which a standardized bacterial suspension was added. Ten inoculated tested strains, was placed in incubators to cultivate for 2-7d and a blank in each strain at the same time. Inhibitory effects were listed in Table-1.

TABLE-1
STRUCTURE AND FEATURE OF TARGET COMPOUNDS

Comp.	R ₁	R ₂	R ₃	Time (h)	Yield (%)	m.p. (°C)
3a	H	H	Cl	10	33.7	237-239
3b	H	H	CH ₃	20	37.0	224-226
3c	CH ₃	H	H	18	38.5	210-211
3d	F	H	H	9.5	31.4	226-228
3e	Cl	H	H	12	29.8	222-224
3f	H	Cl	Cl	13	64.3	233-235
3g	H	H	F	11	32.4	202-203
3h	H	F	CH ₃	12.5	44.2	242-243
3i	H	H	F	7	26.6	232-234
3j	H	H	Br	7	45.1	255-257

RESULTS AND DISCUSSION

In order to verify the effect of the temperature, the compound **2a**, brominatedacetophenone (2 mmol), thiosemicarbazide (2 mmol) and 10 mL ethanol were mixture in a 50 mL round-bottomed flask at different temperature response completely. The results are summarized in Table-2. As shown in Table-2, the reaction under reflux with yield of 31.3 % within 12 h, compared with other conditions reaction time is short, high yield, so the reaction temperature was chosen under

reflux. The formation of newly synthesized compounds was confirmed by recording the ¹H NMR, ¹³C NMR and HRMS. The ¹H NMR spectrum of these compounds showed a singlet at about δ 11.30 corresponding to NH proton. A sharp singlet at around δ 7.35 is attributed to the C-5 proton of the thiazole ring. ¹³C NMR spectrum showed approximately δ 170 is corresponding to C-2 of the thiazole ring. HRMS of these compounds showed intense molecular ion peaks in agreement with their respective molecular formulae, the feature of the target compounds are given in Table-2.

TABLE-2
EFFECT OF REACTION TEMPERATURE ON THE YIELD OF **3a**

Entry	Temperature (°C)	Time (h)	Yield (%)
1	30	26	23.6
2	50	14	25.7
3	Reflux	13	31.3

The compounds were screened for their *in vitro* antifungal activities against ten fungal. The results of antifungal screening reveal that among all the compounds screened three compounds showed moderate antifungal activity. The MIC value of **3** h against two fungal strains *C. neoformans* and *C. albicans* is 8 μg mL⁻¹ respectively. The MIC value of **3i** against two fungal strains *C. neoformans* and *T. mentagrophytes* is 8 μg mL⁻¹ and 16 μg mL⁻¹ respectively and **3a** against *T. mentagrophytes* is 16 μg mL⁻¹, the MIC of others are all beyond 32 μg mL⁻¹.

Conclusion

In conclusion, we have described a simple, a facile protocol for the synthesis of thiazole derivatives in ethanol as reaction medium under reflux. Although antifungal activities of the new thiazole derivatives failed to approach to the MIC of the reference compounds amphotericin B and fluconazole, it could be undertaken further modification in order to obtain new compounds with better antifungal activities.

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