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Synthesis and antifungal activity of (Z)-3-chloromethylenethiochroman-4-ones

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ABSTRACT

A convenient and efficient procedure has been developed for the synthesis of (Z)-3-Chloromethylenethiochroman-4-ones and the chlorination method has not reported before. The chlorination agent, reaction temperature, pressure and reaction time of the chlorination reactions were studied and the probable chlorination mechanism was conjectured. The structures of the novel compounds had been confirmed by ^{1}H-NMR, MS, element analysis and IR spectrum. Their antifungal activity was tested by micro dilution broth susceptibility for ten kinds of fungi. These compounds exhibited potent antifungal activities toward tested fungi.

KEYWORDS

A novel chlorination;
Benzenethiol;
Thiochroman-4-ones;
2-chloroacetyl chloride;
(Z)-3-chloromethylene-thiochroman-4-ones;
Antifungal activity.

INTRODUCTION

Broad spectrum of biological activity of thiochromanone derivatives[1] stimulated extensive studies in the field of thiochromanones chemistry. Some 3-site-substituted thiochromanones were recently shown to be active against fungi; such as 3-bromo[2], 3-mannich bass[1,3,4], 3-enzylidyne[5,6], 4,3-d-triazolo[7] and so on. Chloromethylene substituted at 3-site has not been reported, in this paper three novel (Z)-3-Chloromethylenethiochroman-4-one deriveatives were designed and synthesized by a novel chlorination (2-chloroacetyl chloride was used as chlorinetion agent); their antifungal activity was tested by micro dilution broth susceptibility for ten kinds of fungi.

EXPERIMENTAL

The IR spectra were recorded in KBr on a SHIMADZU FTIR-8400S spectrometer with Fourier transform. The ^{1}H NMR spectra was measured on a Bruker Avance-400 spectrometer from solutions in $CDCl_3$ using TMS (^{1}H) as internal references. The mass spectra were obtained on an Agilent LC-MSD Trap XCT G2446A HPLC-MS spectrometer. Elemental Analysis (C, H, N, S) was realized on Carlo Erba 1106 EA instrument. The progress of reactions was monitored by TLC on silica gel HF254 plates using ethyl acetate-petroleum ether (1:10) as eluent; Spots were observed at 254 nm using ultraviolet lamp or visualized by treatment with iodine vapor. The purity of products was monitored by HPLC using HmethanolH-water (79: 21) as eluent.

3-phenylthiopropanoic acids (I.1, I.2, I.3) (general procedure)

Substituted benzenethiols (50mmol) mixed with the 3-chloropropanoic acid (60mmol) and 50% sodium hydroxide solution (5mL) in a 250mL round bottom flask

under microwave 5-6min. The reactant was cooled to ambient temperature and HCl (1mol/L) was added, a lot of white precipitant were created. The precipitate was filtered off, washed with water and recrystallized from ethanol[6,7]. The yield of product **(I.1-I.3)** is 81-88%.

Thiochroman-4-ones (II.1, II.2, II.3) (general procedure)

Compounds **(I)** (10mmol) mixed with concentrated sulfuric acid (8mL, 98%) at room temperature for 12 h. The concentrated sulfuric acid was diluted by water, collected the solid product and recrystallized from ethanol[6,7]. The yield of product **(II.1-II.3)** is 78-85%.

4-oxothiochroman-3-carbaldehydes (III.1, III.2, III.3) (general procedure)

Ethyl formate (20mmol) and sodium methoxide (40mmol) was dissolved in toluene (50mL), 10mmol of the Substituted thiochromanones II was slowly added at 5°C, and the mixture was stirred for 10 h. The organic phase was extracted three times with water and the combined aqueous phase was acid regulated (pH = 4) with HCl, a lot of yellow precipitant were created. The precipitate was filtered off, washed with water. The yield of product **(III.1-III.3)** is 84-93%.

6-methyl-4-oxothiochroman-3-carbaldehyde (III.1)

Yield: 89%; HPLC: 95%; m.p.: 88-89°C; yellow; solid; APCI-MS *m/z*: $[M]^+$ 207.1; ^{1}H-NMR ($CDCl_3$, 400 MHz): δ = 2.345 (s, 3H), 3.250 (m, 2H),4.202 (m, 2H), 7.211 (m, 2H), 7.354 (s, 1H), 9.628 (d, *J* = 3.650Hz, 1H). Anal. Calcd for $C_{11}H_{10}O_2S$: C 65.01; H 5.76; S 14.75. found: C 64.05; H 4.89; S 15.55.

6-fluoro-7-methyl-4-oxothiochroman-3-carbaldehyde (III.2)

Yield: 91%; HPLC: 96%; m.p.: 91-92°C; yellow; solid; APCI-MS *m/z*: $[M]^+$ 224.9; ^{1}H-NMR ($CDCl_3$, 400 MHz): δ = 2.299 (d, *J* = 1.360Hz, 3H), 3.195 (m, 2H), 4.104 (m, 2H), 7.106 (d, *J* = 3.480Hz, 1H), 7.361 (s, 1H), 9.598 (d, *J* = 4.012Hz, 1H). Anal. Calcd for $C_{11}H_9FO_2S$: C 59.28; H 4.35; S 14.08. found: C 58.92; H 4.05; S 14.30.

6-chloro-4-oxothiochroman-3-carbaldehyde (III.3)

Yield: 85%; HPLC: 92%; m.p.: 103-104°C; yellow; solid; APCI-MS *m/z*: $[M]^+$ 226.65; ^{1}H-NMR ($CDCl_3$, 400 MHz): 3.209 (m, 2H),4.218 (m, 2H), 7.356 (d, *J* = 5.907Hz, 1H), 7.665 (t, *J* = 9.417Hz, 2H), 9.651 (d, *J* = 3.524Hz, 1H). Anal. Calcd for $C_{10}H_7ClO_2S$: C 53.45; H 3.24; S 13.15 found: C 52.99; H 3.11; S 14.15.

(Z)-3-chloromethylenethiochroman-4-ones (IV.1, IV.2, IV.3) (general procedure)

Compounds **(III)** (10mmol) and 2-chloroacetyl chloride (15mmol) was dissolved in dichloromethane (40mL) in a sealed tube (100mL), and the mixture was stirred at 50°C for 2 h. The organic phase was washed twice with water and dried dichloromethane under reduced pressure, a lot of yellow solid were created. The solid was purified by silicagel column chromatography, eluting with dichloromethane: petroleum ether = 1: 10(v/v). The yield of product **(IV.1, IV.2, IV.3)** is 74-89%.

(Z)-3-chloromethylene-6-methylthiochroman-4-one (IV.1)

Yield: 76%; HPLC: 95%; m.p.: 64-65°C; yellow; solid; IR spectrum(KBr), ν: 1585.38, 1600.81, 1658.67 cm^{-1}; APCI-MS *m/z*: $[M]^+$ 224.1; ^{1}H-NMR ($CDCl_3$, 400 MHz): δ = 2.355 (s, 3H), 4.002 (d, *J* = 0.800Hz, 2H), 7.211 (m, 2H), 7.354 (s, 1H), 7.948 (s, 1H). Anal. Calcd for $C_{11}H_9ClOS$: C 58.80, H 4.04, S 14.27. found: C 58.86, H 3.94, S 14.19.

(Z)-3-chloromethylene-7-fluoro-6-methylthiochroman-4-one (IV.2)

Yield: 85%; HPLC: 96%; m.p.: 65-67°C; yellow; solid; IR spectrum(KBr), ν: 1587.31, 1606.59, 1660.60 cm^{-1}; APCI-MS *m/z*: $[M]^+$ 242.1; ^{1}H-NMR ($CDCl_3$, 400 MHz): δ = 2.299 (d, *J* = 1.360Hz, 3H), 4.000 (d, *J* = 0.920Hz, 2H), 7.106 (d, *J* = 3.480Hz, 1H), 7.361 (s, 1H), 7.759 (d, *J* = 10.000Hz, 1H). Anal. Calcd for $C_{11}H_8ClFOS$: C 54.44, H 3.32, S, 13.21. found: C 54.53, H 3.28, S 13.17.

(Z)-6-chloro-3-chloromethylenethiochroman-4-one (IV.3)

Yield: 79%; HPLC: 92%; m.p.: 83-84°C; yellow; solid; IR spectrum(KBr), *v*: 1581.52, 1662.52 cm^{-1}; APCI-MS *m/z*: $[M]^+$ 244.1; ^{1}H-NMR ($CDCl_3$, 400 MHz): δ = 4.022 (d, *J* = 0.952Hz, 2H), 7.269 (d, *J* =

6.608Hz, 1H), 7.375 (t, *J* = 10.644Hz, 2H), 8.101 (d, *J* = 2.352Hz, 1H). Anal. Calcd for $C_{10}H_6Cl_2OS$: C 49.00, H 2.47, S 13.08. found: C 49.07, H 2.41, S 13.07.

Antifungal activity in vitro

The antifungal activity of thiochromanones **(III.1, III.2, III.3,IV.1, IV.2, IV.3)** was compared with amphotericin B from known micro dilution broth susceptibility test method. The stock solutions of conjugates viz. **(III.1, III.2, III.3,IV.1, IV.2, IV.3)** along with amphotericin B were prepared in DMSO. The stock solution of each of these compounds was serially diluted (64.0, 32.0, 16.0, 8.00, 4.00, 2.00, 1.00, 0.500, 0.250, 0.125 mgL^{-1}) and added to RPMI1640 HmediumH, after which a standardised bacterial suspension was added.

Antifungal activity test in vitro was done on multiresistant fungi specially causing infections in human being, for example, C. albicas, C. tropicalis, C. neoformans, E. floccosum, M. gypseum, A. niger, S. schenekn, C. Krusei, C. parapsilosis, C. glabrata. The results have been tabulated (see TABLE 1).

There were encouraging results revealed on the antifungal activities. All (Z)-3-chloromethylenethiochroman-4-one derivateives especially **(IV.1)** exhibited potent antifungal activities toward C. neoformans, and had selective activity against other tested fungis for instance **(IV.1)** toward C. glabrata, C. Krusei and M. gypseum; **(IV.2)** toward C. neoformans, M. gypseum and C. parapsilosis; **(IV.3)** toward C. albicas, C. tropicalis, C. neoformans, E. floccosum, S. schenek and C. parapsilosis also exhibited potent antifungal activities. But three compounds had no significant activity against A. Niger. All 4-oxothiochroman-3-carbaldehydes also exhibited antifungal activities towards tested fungis, but their activities was not as potent as (Z)-3-chloromethylene-thiochroman-4-one derivateives.

TABLE 1 : Minimum inhibitory concentration (MIC), correlation diagram (in mgL^{-1}) of compounds against fungi strain.

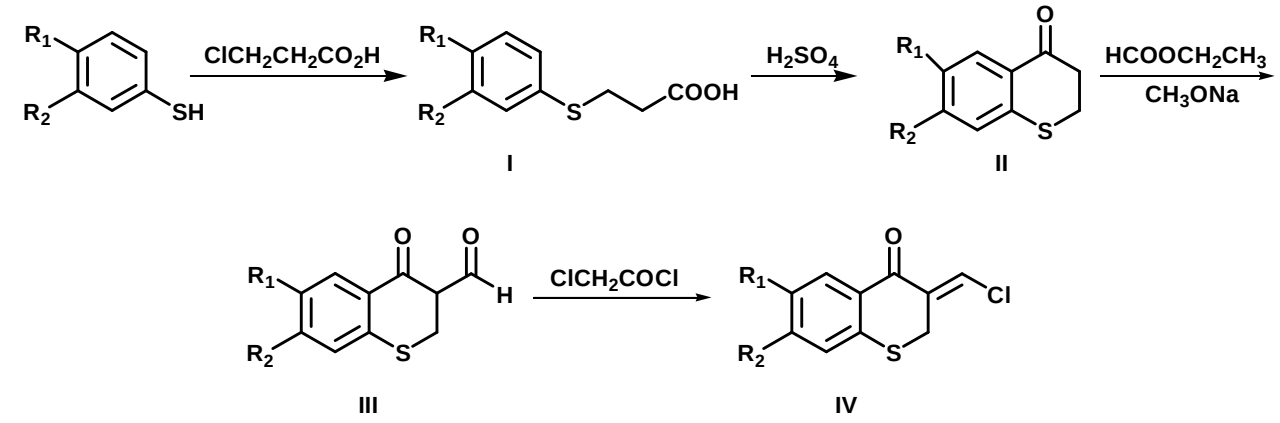
Fungi	Compounds							
	III.1	III.2	III.3	IV.1	IV.2	IV.3	AmB	DMSO (v/v)
C. albicas	32.0	64.0	64.0	8.00	32.0	4.00	0.500	----
C.tropicalis	32.0	64.0	32.0	32.0	16.0	4.00	8.00	----
C. neoformans	64.0	16.0	32.0	0.500	1.00	4.00	1.00	----
E. floccosum	32.0	64.0	64.0	----	32.0	2.00	1.00	----
M. gypseum	32.0	32.0	32.0	4.00	4.00	16.0	16.0	----
A. niger	64.0	32.0	----	----	----	----	1.00	----
S. schenek	16.0	64.0	64.0	8.00	16.0	4.00	2.00	----
C. Krusei	32.0	32.0	64.0	4.00	8.00	32.0	16.0	----
C. parapsilosis	64.0	32.0	16.0	8.00	4.00	2.00	1.00	----
C. glabrata	32.0	----	32.0	2.00	8.00	16.0	2.00	----

(-----) No activity.

RESULTS AND DISCUSSION

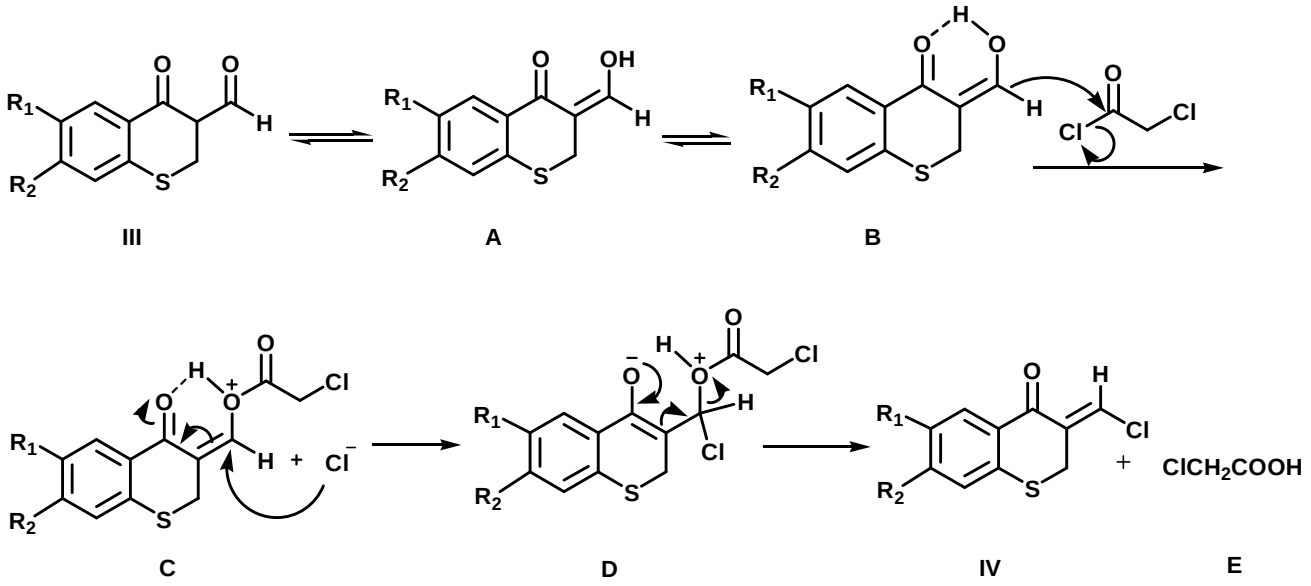
The goal of the present work was to find conditions for the preparation of (Z)-3-chloromethylenethiochroman-4-ones and study their antifungal activity. We used a reported convenient procedure for the synthesis of thiochromanones **(II)** by microwave-H_2SO_4 heterocyclization of accessible benzenethio with 3-chloropropanoic acid[8,9]. A hydrogen atom in position 3 of the thiochromanones is capable of being replaced by formyl group, used reactions with ethyl formate and sodium methoxide in toluene to give the corresponding 4-oxothiochroman-3-carbaldehydes **(III)** as a result of ester condensation. The 4-oxothiochroman-3-carbaldehydes **(III)** reacted with 2-chloroacetyl chloride in dichloromethane to give the corresponding (Z)-3-chloromethylenethiochroman-4-ones **(IV)** as a result of an unreported HsimpleH and efficient chlorination. Figure 1 is the synthetic route of target compounds. The antifungal activity of (Z)-3-chloromethy-lenethiochroman-4-ones **(IV.1, IV.2, IV.3)** was compared with amphotericin B from known micro dilution broth susceptibility test method. The lowest concentration of (Z)-3-chloromethylene-thiochroman-4-ones in mgL^{-1} that prevented in vitro growth of fungi has been represented as MIC (minimum inhibitory concentration) shown in TABLE 1. Laboratory experiments showed that (Z)-3-chloromethylenethiochroman-4-ones **(IV)** exhibits an effect in against fungi; for instance, it exhibited potent activity of against *C. neoformans*.

We used dichloromethane as solvent for chlorination of the 3-carbaldehyde group in 4-oxothiochroman-3-carbaldehydes **(III)** by the action of 2-chloroacetyl chloride, acetyl chloride or thionyl chloride. 4-oxothiochroman-3-carbaldehydes **(III)** reacted with the 2-chloroacetyl chloride at a ratio of 1: 1.5 in



I.1, II.1, III.1, IV.1 (R_1=CH_3, R_2=H); I.2, II.2, III.2, IV.2 (R_1=CH_3, R_2=F); I.3, II.3, III.3, IV.3 (R_1=Cl, R_2=H)

Figure 1 : The synthetic route of target compounds.



I.1, II.1, III.1, IV.1 (R_1=CH_3, R_2=H); I.2, II.2, III.2, IV.2 (R_1=CH_3, R_2=F); I.3, II.3, III.3, IV.3 (R_1=Cl, R_2=H)

Figure 2 : A probable chlorination mechanism.

dichloromethane, the yield of products **(IV)** increased from 41-56% (room temperature, atmospheric, 18h) to 76-85% (50°C, sealed tube, 2h). Compounds **(III)** reacted with acetyl chloride or thionyl chloride also at a ratio of 1: 1.5 in sealed tube, the solution was stirred for 2h at 50°C, the yield of corresponding products **(IV)** is 36-49% or 61-70%, so 2-chloroacetyl chloride was selected as chlorination agent.

A probable chlorination mechanism is illustrated by figure2. It involves tautomeric transformation of the aldehyde form of 4-oxothiochroman-3-carbaldehydes **(III)** into enol form **(A)** which form hydrogen bond between hydroxyl group and carbonyl group to give intermediate **(B)** having activated nucleophilic oxygen center. Attack by the oxygen atom on the C = O carbon atom of 2-chloroacetyl chloride gives structure **(C)** and chloride ion. The β-carbon atom at the conjugated double bond is attacked by chloride ion to give structure **(D)** which is stabilized by electron transfer and stripped carboxylic acids to give structure **(IV)**.

CONCLUSION

The aim of this study was to develop an efficient synthetic approach to construct various (Z)-3-chloromethylenethiochroman-4-ones and to screen for

possible antifungal activities. The novel chlorination of the 4-oxothiochroman-3-carbaldehydes reacted with 2-chloroacetyl chloride is a simple and efficient HmethodH, it shorten reaction time, not require Hhigh temperatureH, simplified synthesis process and enhance product yield .The efficient synthetic approach disclosed herein had lead to quick output of a series of (Z)-3-chloromethylenethiochroman-4-ones for the evaluation of antifungal activities. There were encouraging results revealed on the antifungal activities. All derivatives especially compounds **(IV.1)** and **(IV.2)** as the potent inhibitor for C. neoformans, and had selective activity against other tested fungi but had no significant activity against A. niger.

The conditions for the preparation of (E)-3-chloromethylenethiochroman-4-ones and their antifungal activity are still in study.

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