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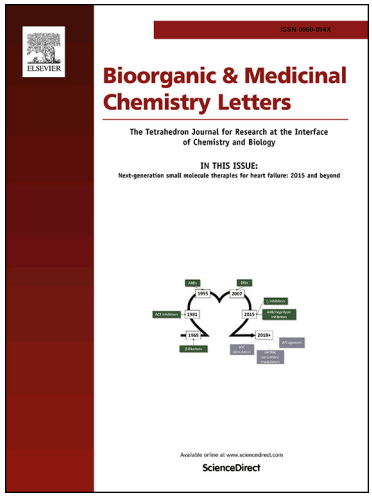

Design, synthesis, and biological evaluation of 4-chloro-2*H*-thiochromenes featuring nitrogen-containing side chains as potent an agents

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Design, synthesis, and biological evaluation of 4-chloro-2*H*-thiochromenes featuring nitrogen-containing side chains as potent an agents

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Abstract

A series of 4-chloro-2*H*-thiochromenes featuring nitrogen-containing side chains were designed, synthesized and tested *in vitro* for their antifungal activities. The results of preliminary antifungal tests showed that most target compounds exhibited good inhibitory activities against *Candida albicans*, *Cryptococcus neoformans*, *Candida tropicalis*. Notably, compounds **10e** and **10y** showed most potent activity *in vitro* against a variety of fungal pathogens with low MICs. Meanwhile, low cytotoxicity on mammalian cells has been observed for compounds **10e** and **10y** in the tested concentrations by the MTT assay. Therefore, the 4-chloro-2*H*-thiochromenes with nitrogen-containing groups provide new lead structures in the search for novel antifungal agents.

Keywords: 4-Chloro-2*H*-thiochromenes; Antifungal activity; Nitrogen-containing group; Cytotoxicity

Invasive fungal infections (IFIs) present serious public health concerns and the incidence of their associated mortality has been increasing dramatically, especially in immunocompromised patients, patients with acquired immune deficiency syndrome (AIDS), and patients undergoing cancer chemotherapy.[1,2] *Cryptococcus*, *Candida*, *Aspergillus*, and *Pneumocystis* account for a large percentage of the main pathogens that lead to the deaths of invasive fungal infections.[3] Several classes of antifungal agents such as polyenes, azoles, echinocandins are widely used clinically, but some available antifungal drugs just have

modest effects on the treatment of invasive fungal infections caused by *Candidiasis* and *Cryptococcosis*.[4] There is still an urgent need to develop a new generation of antifungal agents with novel mechanism of action to meet the increasing requirements for managing the fungal infections.

Thiochroman-4-ones which are analogs of chromones possess several biological activities, and serve as building blocks in synthetic chemistry.[5] Since 1980s, thiochroman-4-one derivatives with good antifungal activities were reported by the structural modification. A substituent at 3-position of thiochroman-4-ones can effectively increase their antifungal activities. Nakib et al.[6] reported that 3-benzylidenethiochroman-4-ones (**1, Fig. 1**) exhibited considerable antifungal activities against *Candida* species, *Cryptococcus* strains, *Torulopsis glabrata* and *Trichosporon cutaneum*. In our previous works,[7,8] a series of 3-substituted thiochroman-4-one derivatives such as 3-mannich bases (**2, Fig. 1**), 3-bromos (**3, Fig. 1**) were synthesized and showed antifungal activities against *C. albicans*, *C. neoformans*, *E. floccosum*, *T. rubrum*, and *M. gypseum*. Based on results obtained from structure-activity relationships (SARs) of thiochroman-4-one derivatives,[6-9] the thiochroman-4-one attached a nitrogen-containing moiety increase antifungal activity to some extent. In particular, 3-substituted thiochroman-4-one analogues such as 3-mannich bases, exhibited better antifungal activities.[8] Thiochromanone moieties found in 3- substituted analogs have shown in **Fig. 1**.

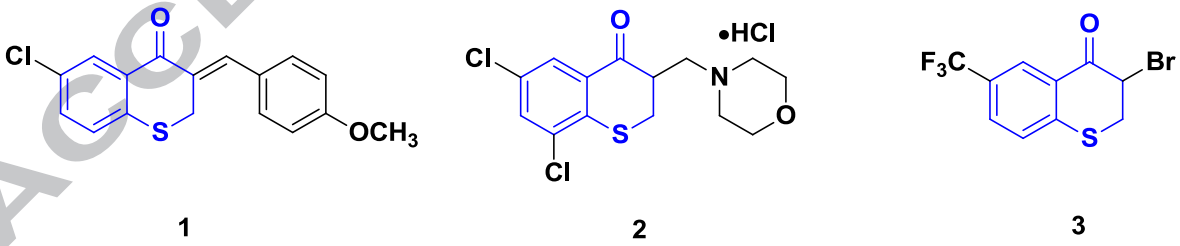


Fig. 1. The structures of 3-substituted thiochroman-4-one derivatives

Compounds 4-chloro-2*H*-thiochromene-3-carbaldehydes have been identified as modest antifungal agents against a varieties of pathogens in our lab (not published). When the carbonyl groups were substituted with chloro groups of thiochroman-4-ones, it appears that position 4 were tolerable. (Data shown in **Fig. 2** and **Table 1**). This work is a continuation of

our study on the chemical modification and biological evaluation of 4-chloro-2*H*-thiochromene-3-carbaldehydes.

Aliphatic amine moieties (e.g., diethylamine, piperidine, pyrrolidine) are important parts of medicinal compounds with various biological properties such as anti-Alzheimer's disease,[10] anticancer[11] and antifungal activities.[12] In addition, azole moieties are important pharmacophores in drug design[13] and widely distributed in antifungal agents such as voriconazole, fluconazole, and itraconazole.[14] Although azoles are the most widely used antifungals as first-line antifungal therapy, the search for novel azoles with broadest activity, low toxicity, and low resistance still remains an attractive subject.[15] The widespread applications of azole and aliphatic amine moieties in medicinal chemistry encourage us to explore more excellent antifungals of 4-chloro-2*H*-thiochromenes featuring these fragments. Thus, we designed and synthesized a new series of compounds by incorporating 4-chloro-2*H*-thiochromene-3-carbaldehydes with a nitrogen-containing side chain in a single molecule (**Fig. 2**).

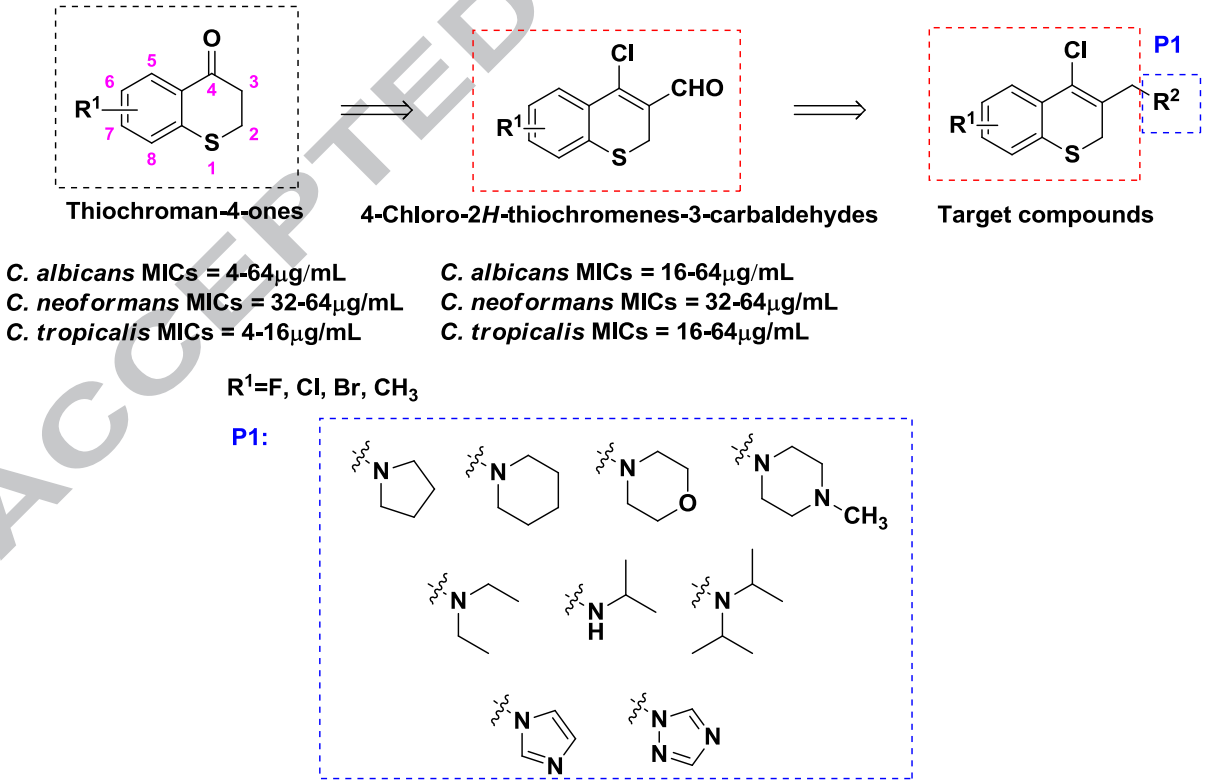
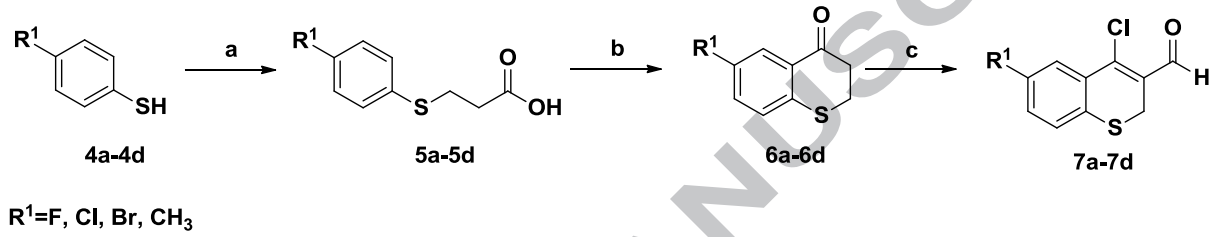


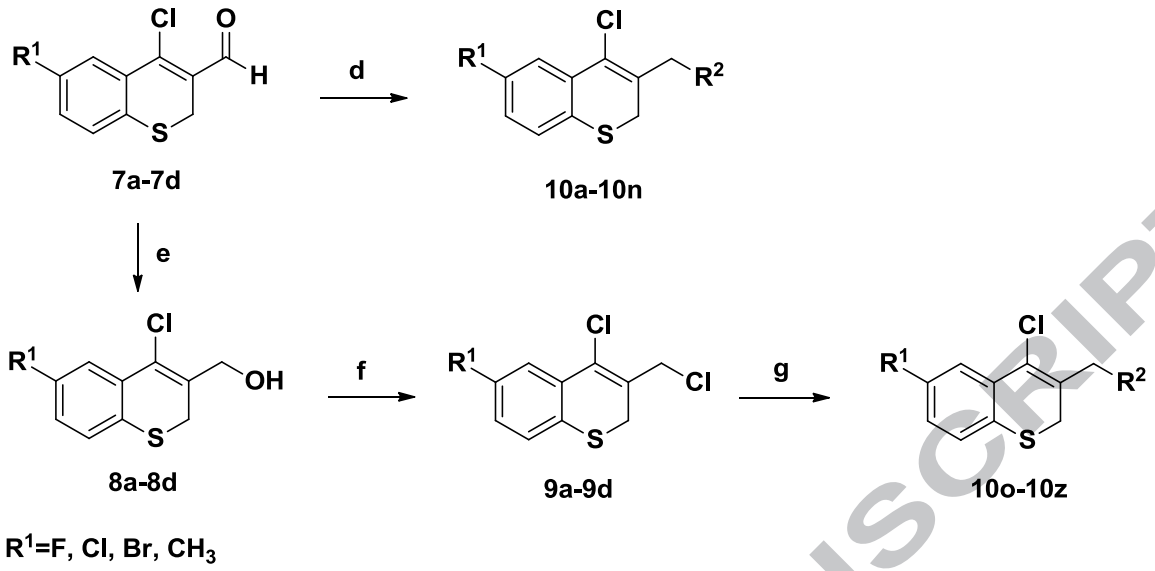
Fig. 2. Rationale design of the title compounds

Thichroman-4-ones **6a-6d**[7] and the key intermediates 4-chloro-2*H*-thiochromene-3-carbaldehydes **7a-7d**[16] were synthesized according to literature procedures (**Scheme 1**). Firstly, compounds **5a-5d** were obtained by reactions of commercially available substituted thiophenols **4a-4d** and *β*-chloropropionic acid, and then were cyclized in the presence of concentrated H_2SO_4 to afford thiochroman-4-ones **6a-6d** *via* intramolecular Friedel-Crafts. Subsequently, ketones **6a-6d** were under Vilsmeier–Haack conditions to generate the key intermediates **7a-7d**.



Scheme 1. Reagents and conditions: (a) (i) KOH, H_2O, EtOH, 60 °C; (ii) *β*-chloropropionic acid, 30% K_2CO_3 aqueous solution, reflux; (b) H_2SO_4; (c) $POCl_3$, DMF, 50 °C.

The synthesis of target compounds **10a–10z** were illustrated in **Scheme 2**. Aldehydes **7a-7d** and amines (diethylamine, piperidine, pyrrolidine, N-methyl piperazine) were treated with $NaBH(OAc)_3$ in 1,2-dichloroethane at room temperature to give compounds **10a-10n** *via* reductive aminations.[17] Aldehydes **7a-7d** were treated with $NaBH_4$ to afford alcohols **8a-8d**, which were then chlorinated with $SOCl_2$ to afford compounds **9a-9d**. Subsequently, nucleophilic substitutions of compounds **9a-9d** with aliphatic amines (diisopropylamine and isopropylamine) or azoles (imidazole and 1,2,4-triazole) at the presence of K_2CO_3/KI in DMF gave **10o-10z**.



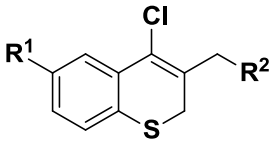
Scheme 2. Reagents and conditions: (d) $NaBH(OAc)_3$, amine, DCE, N_2, rt. (e) $NaBH_4$, THF, rt; (f) $SOCl_2$, $CHCl_2$, reflux; (g) aliphatic amine/azole, K_2CO_3, KI, DMF, 50 ^oC.

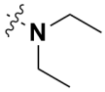
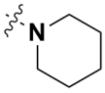
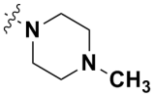
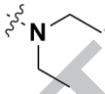
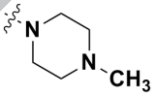
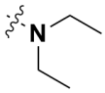
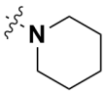
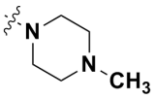
The compounds **10a-10z**, **6a-6d, 7a-7d** have been screened for their antifungal activities against *Candida albicans* (cpcc 400616), fluconazole-sensitive strains of *Candida albicans* (ATCC SC5314), fluconazole-resistant strains of *Candida albicans* (strain 103), *Cryptococcus neoformans* (cgmcc 2.3161), *Candida tropicalis* (cgmcc 2.3739) and *Aspergillus fumigatus* (cgmcc 3.7795). According to the National Committee for Clinical Laboratory Standards (NCCLS), the minimal inhibitory concentrations (MICs) of target compounds were determined by the broth microdilution method in 96-well plates.[18] Fluconazole (FCZ) was used as reference drug. In brief, tested compounds and fluconzole were dissolved in DMSO, respectively. Then, these compounds serially diluted by growth medium in 96-well microtest plates. The inoculum suspension was added to each well of microtiter plate to give the concentrations (128, 64, 32, 16, 8, 4, 2, 1, 0.5, 0.25 μg/mL) and incubated at 32^{o}C. MIC values were determined by visual observation after 2-7d of incubation.

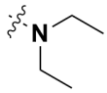
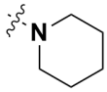
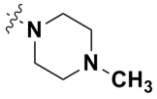
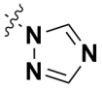
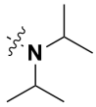
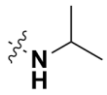
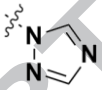
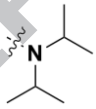
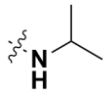
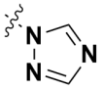
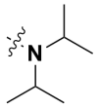
The *in vitro* antifungal activities are summarized in **Table 1**. Most of the synthesized compounds exhibited moderate to good antifungal activities. Among these compounds, compound **10e** and **10y** showed comparable or even better efficacy than fluconazole against all tested strains, particularly for *C. albicans* (MICs = 0.5-8 μg/mL) and *C. neoformans* (MIC = 0.25-1 μg/mL).

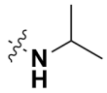
Table 1

In vitro antifungal activities of the target compounds (MIC, μg/mL)[a].



Compd.	R^1	R^2	MIC					
			C. a (I)	*C. a* (II)	*C. a* (III)	*C. n*	*C. t*	*A. f*
10a	F		8	8	64	2	2	>128
10b	F		2	8	16	2	16	>128
10c	F		8	8	64	4	16	>128
10d	F		16	16	16	1	32	>128
10e	Cl		4	0. 5	8	1	2	>128
10f	Cl		32	1	>128	4	4	>128
10g	Cl		32	16	64	4	64	>128
10h	Br		16	8	64	4	64	>128
10i	Br		32	8	64	4	128	>128
10j	Br		16	8	64	8	128	>128
10k	Br		16	8	64	8	16	>128

10l	H_3C		32	32	64	8	64	>128
10m	H_3C		32	16	128	16	64	>128
10n	H_3C		32	8	64	32	8	>128
10o	F		16	4	64	32	8	>128
10p	F		16	4	32	64	8	>128
10q	F		4	4	64	4	2	>128
10r	F		8	2	64	4	2	64
10s	Cl		32	16	64	8	64	64
10t	Cl		16	8	32	4	32	>128
10u	Cl		4	4	64	64	64	>128
10v	Cl		32	16	32	0.5	32	64
10w	Br		64	16	>128	8	>128	>128
10x	Br		>128	64	>128	8	>128	>128
10y	Br		0.5	0.5	0.5	0.25	>128	>128

10z	Br		8	4	16	4	>128	>128
6a	F	-	32	8	64	64	16	64
6b	Cl	-	16	4	16	32	8	>128
6c	Br	-	32	8	64	64	16	>128
6d	CH_3	-	16	8	64	32	4	>128
7a	F	-	64	64	64	32	16	>128
7b	Cl	-	64	32	64	32	32	>128
7c	Br	-	32	16	64	32	64	>128
7d	CH_3	-	32	16	64	64	64	>128
FCZ	-	-	4	1	32	1	2	>128

[a]Abbreviations: *C. a* (I): *Candida albicans* (cpcc 400616); *C. a* (II): *Candida albicans* (ATCC SC5314); *C. a* (III): strain 103, fluconazole-resistant strains of *Candida albicans*; *C. n*: *Cryptococcus neoformans* (cgmcc 2.3161); *C. t*: *Candida tropicalis* (cgmcc 2.3739); *A. f*: *Aspergillus fumigatus* (cgmcc 3.7795); FCZ: fluconzole.

The *in vitro* antifungal activities of compounds **10a-10z** are summarized in **Table 1**, and the SARs of these compounds were also discussed.

Firstly, the introduction of acyclic aliphatic amine moieties on the 3-position of **7a–7d** afforded more potent compounds (eg., **10a**, **10e**) than those (eg., **10d**, **10g**) containing the cyclic aliphatic amine moieties on the 3-position. Secondly, compounds (e.g., **10w-10x**) with imidazole or 1,2,4-triazole moieties showed lower activities than the compounds (e.g., **10y-10z**) with aliphatic amine moieties. Meanwhile, the relationship between various substituents on the 6-position of benzene ring were investigated. These compounds showed broad antifungal activities when 6-position of the benzene ring was substituted with a fluoro (e.g., **10a-10c, 10q-10r**) or chloro group (e.g., **10e-10f**). On the contrary, when 6-position was substituted with an electron-donating group such as methyl (e.g., **10l-10n**), the antifungal

activities markedly decreased. Moreover, most of the target compounds showed improved antifungal activities against tested fungal strains compared with the intermediates **6a-6d** and **7a-7d**. It can be seen that 4-chloro-2*H*-thiochromenes attached a nitrogen-containing side chain, their antifungal activities obviously increased. This finding demonstrates that the 3-position of 4-chloro-2*H*-thiochromenes attached a nitrogen-containing side chain can effectively enhance antifungal activities.

In order to ensure selectivity of the antifungal effects, the cytotoxicity of compounds **10e** and **10y** were evaluated by MTT assay using four mammalian cell lines, including A549 (human lung carcinoma), HepG2 (human liver carcinoma), HT-29 (human colorectral cancer) and H9C2 (normal, rat myocytes). As presented in **Table 2**, the IC_{50} values of compound **7b** were 21.53 μg/mL in HepG2 and 18.44 μg/mL in HT-29, respectively. Compound **7c** gave IC_{50} values of 26.61 μg/mL against HepG2 and 28.79 μg/mL against HT-29. Compound **10e** and **10y** showed low toxicities in four cell lines in tested concentrations. We concluded that introduction of nitrogen-containing side chain at the 3-position of 4-chloro-2*H*-thiochromenes favorably reduces its cytotoxic activities.

Table 2 Cytotoxicity of tested compounds on mammalian cells (IC_{50}, μg/mL).

	A549	HepG2	HT-29	H9C2
10e	>100	>100	>100	>100
10y	36.04	>100	>100	>100
7b	>100	21.53	18.44	>100
7c	>100	26.61	28.79	>100
FCZ	>100	>100	>100	>100
DOX	1.77	2.90	1.44	0.29

In conclusion, we have synthesized a series of 4-chloro-2*H*-thiochromenes featuring nitrogen-containing side chains as antifungal agents. Among these compounds, compounds **10e** and **10y** exhibited excellent antifungal activities against tested fugal strains, and no cytotoxic activities against mammalian cells. The investigation of antifungal mechanism of these compounds are underway.

Acknowledgements

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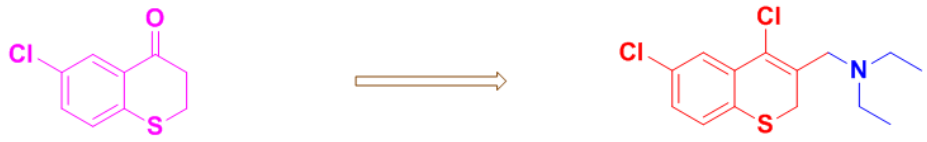

C. albicans (cpcc 400616) MIC = 16 μg/mL
C. albicans (ATCC SC5314) MIC = 4 μg/mL
Strain 103 MIC= 16 μg/mL
C. neoformans (cgmcc 2.3161) MIC = 32 μg/mL
C. tropicalis (cgmcc 2.3739) MIC = 8 μg/mL

C. albicans (cpcc 400616) MIC = 4 μg/mL
C. albicans (ATCC SC5314) MIC = 0.5 μg/mL
Strain 103 MIC= 8 μg/mL
C. neoformans (cgmcc 2.3161) MIC = 1 μg/mL
C. tropicalis (cgmcc 2.3739) MIC = 2 μg/mL

Highlights

1. Twenty-six 4-chloro-2*H*-thiochromenes were designed and synthesized.
2. Most compounds showed good antifungal activities against tested fungal strains.
3. Compound **10e** and **10y** showed better efficacy than fluconazole against strain 103.