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Original article

Design, synthesis and biological evaluation of novel non-azole derivatives as potential antifungal agents

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ABSTRACT

A series of 3-substituted quinazolinones, 2-substituted quinoxalines and 2-substituted benzopyrans were synthesized and evaluated for their antifungal activity *in vitro*. The new compounds revealed excellent *in vitro* antifungal activity with broad spectrum. The structure–activity relationships (SARs) of the derivatives were analyzed. Compound **9A2** exhibits better antifungal activity against 5 tested fungi *in vitro* than fluconazole, especially against *Trichophyton rubrum* and *Microsporum gypseum*. This study provides a series of novel lead compounds for the development of non-azole antifungal agents.

1. Introduction

During the past several years, the incidence of life-threatening invasive and systemic fungal infections has increased dramatically due to an increase in the number of immunocompromised hosts, such as patients undergoing anticancer chemotherapy or organ transplants and patients with AIDS [1,2]. Several antifungal drugs such as azoles, amphotericin B [3], 5-fluorocytosine [4], and caspofungin [5] have been developed to reduce the impact of fungal diseases (Fig. 1). Azoles, especially triazole agents, are used widely and effectively as first-line agents for the treatment of fungal infections in the clinic. However, the increasing administration of antifungal agents has led to fungal resistance [6,7], and the currently available antifungal drugs do not meet the requirements of clinical treatments for complex infections. Therefore, new antifungal drugs are a major focus of drug development.

Lanosterol 14a-demethylase (CYP51) is a key enzyme in fungal sterol biosynthesis [8]. Azole antifungals are competitive inhibitors of CYP51 that are used clinically [9]; however, cases of fatal hepatotoxicity with azole drugs have been reported due to the coordination of azoles with the heme groups of cytochrome P450 enzymes [10]. Therefore, the search for new non-azole CYP51 inhibitors is important, and this area has been a focus of our research in recent years. Three-dimensional models of CYP51 have been constructed using the homology modeling technique [11–13]. Among our reported non-azole lead compounds, the molecules based on the isoquinoline scaffold showed potent antifungal activity, and L-6 was the most potent compound that interacted with the residues of fungal CYP51 apoprotein without binding to the heme (Figs. 1 and 2) [14,15]. A series of compounds were designed and synthesized to investigate the structure–activity relationships of non-azole antifungal compounds. First, we replaced the isoquinoline scaffold with quinazolinone, benzopyran or quinoxaline in order to investigate the antifungal activity of the core scaffold structure. A long alkyl side chain was introduced at the position 3 of the quinazolinone, the position 2 of the quinoxaline or the benzopyran ring, in order to interact with the hydrophobic S3 pocket of CYP51. As a result, a series of 3-substituted quinazolinone compounds, 2-substituted quinoxaline compounds and 2-substituted benzopyran compounds (Fig. 2) were designed and synthesized, and their antifungal activity was evaluated *in vitro*.

2. Experimental

The chemical synthesis of heterocyclic compounds is outlined in Schemes 1–3. First, treatment of substituted 2-aminobenzoic

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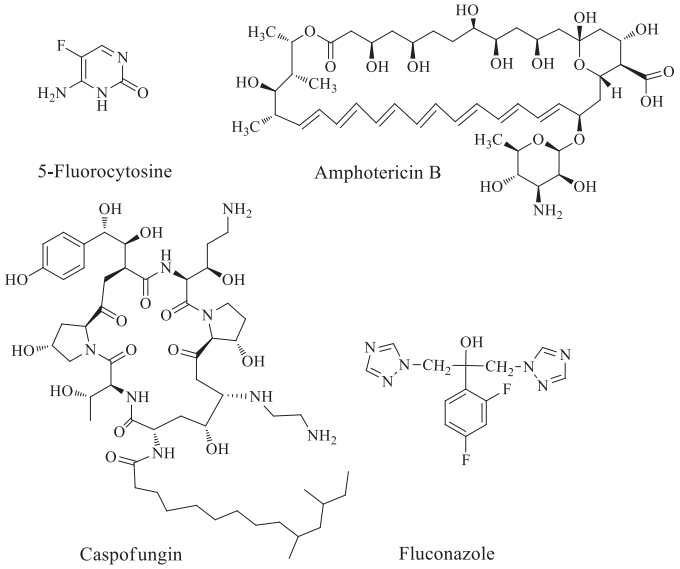


Fig. 1. Antifungal drugs.

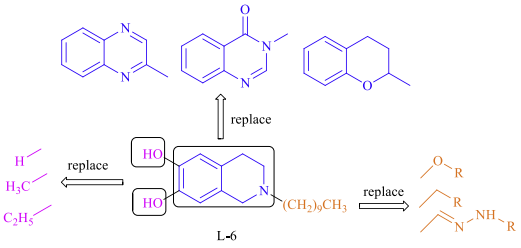


Fig. 2. The targeted compounds.

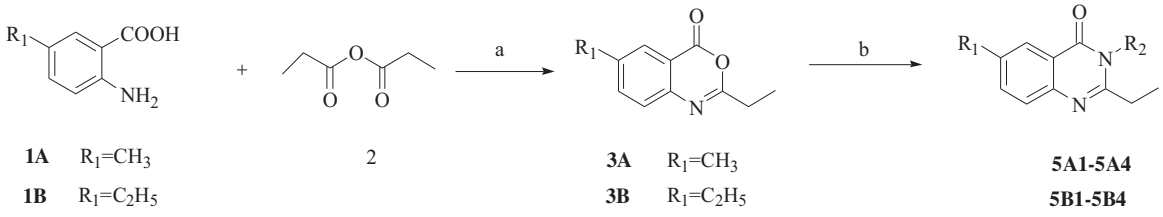
acid **1A**–**1B** with propionic acid anhydride **2** afforded compounds **3A**–**3B** [16–18]. Then, intermediates **3A**–**3B** were reacted with different alkylamines in dry dimethylformamide (DMF) to afford the targeted compounds **5A**–**5B**. Compounds **8A1**–**8A5** were synthesized according to a reported procedure [19,20] by heating ethanolic mixtures to 160–170 °C or using microwave (MW) irradiation for 1 h in the presence of diisopropylamine (DIPA) as a base. Reduction of **8A1**–**A5** with lithium aluminum hydride ($LiAlH_4$) in an ice bath yielded the targeted compounds **9A1**–**A5**.

In order to generate useful intermediates, condensation of 1,2-diaminobenzene **10** with pyruvaldehyde gave 2-methylquinoxaline **11** in good yields [21]. A good method for preparing the quinoxaline-2-carboxaldehyde **12** was found in carrying out the oxidation with selenium dioxide of compound **11** in aqueous dioxane at moderate temperatures [22]. Condensation of compound **12** with hydrazines resulted in corresponding hydrazone compounds **13A1**–**13A4** [23]. All chemical structures were confirmed by electrospray ionization mass spectrometry and nuclear magnetic resonance spectroscopic analyses.

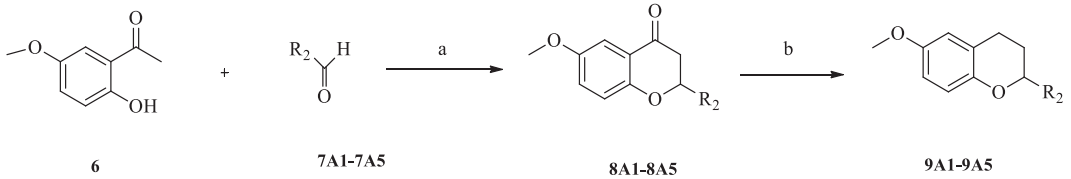
In vitro antifungal activity was measured by means of the MIC using the serial dilution method in 96-well microtest plates. Test fungal strains were obtained from the ATCC. The MIC determination was performed according to the National Committee for Clinical Laboratory Standards (NCCLS) recommendations with RPMI 1640 (Sigma) buffered with 0.165MMOPS (Sigma) as the test medium [24]. The MIC value was defined as the lowest concentration of test compounds that resulted in a culture with turbidity less than or equal to 80% inhibition when compared with the growth of the control. Test compounds were dissolved in DMSO serially diluted in growth medium. The yeasts were incubated at 35 °C and the dermatophytes at 28 °C Growth MIC was determined at 24 h for Candida species, at 72 h for *Cryptococcus neoformans*, and at 7 days for filamentous fungi.

3. Results and discussion

The antifungal activity of targeted compounds was listed in Table 1. Most targeted compounds exhibited antifungal activity against the five tested fungi. Compounds **8**, **9** and **10** showed broad potency against *Candida tropicalis, Trichophyton rubrum* and *Microsporum gypseum*. Compounds **8**, **9** and **10** were 2 to 60-folds more potent against *C. tropicalis* than fluconazole with their MIC_{80} values in the range of 0.24–7.21 μmol/L. Compound **9A2** showed a better antifungal activity against the 5 tested fungi *in vitro* than fluconazole, especially against *T. rubrum* and *M. gypseum*. On the *C. neoformans* strain, compounds **8A1**, **8A2**, **8A4**, **8A5**, **9A1**–**9A4** and **13A3** were more active than fluconazole. Compound **5** exhibited weaker antifungal activity than the other compounds against the five tested fungi. Compound **5A2** was active against *C. tropicalis* at a concentration of 8.72 μmol/L. Compounds **8A** and **9A** were more active than fluconazole against the 5 tested fungi except *Candida*



Scheme 1. The synthesis route of the target compounds. Reagents and conditions: (a) 120 °C, reflux, yield 61.6%; (b) alkylamine, *N*,*N*′-dicyclohexylcarbodiimide (DCC), dimethylformamide (DMF), 16 h, yield 30%–60%.



Scheme 2. The synthesis route of the target compounds. Reagents and conditions: (a) Appropriate aldehyde, diisopropylamine (DIPA), ethanol (EtOH), microwave (MW), 160–170 °C, 1 h, 22%–33%; (b) lithium aluminum hydride ($LiAlH_4$), tetrahydrofuran (THF), ice bath, yield 65%–78%.