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Preliminary communication

Discovery of highly potent triazole antifungal derivatives by heterocycle-benzene bioisosteric replacement

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ABSTRACT

On the basis of our previously discovered triazole antifungal lead compounds, heterocycle-benzene bioisosteric replacement was used to improve their pharmacokinetic profile. The designed new triazole derivatives have good antifungal activity toward a wide range of pathogenic fungi. Their binding mode with the target enzyme was clarified by molecular docking. The MIC value of the highly potent compound **8f** against *Candida albicans*, *Candida tropicalis*, and *Cryptococcus neoformans* is 0.016 μg/mL, 0.004 μg/mL, and 0.016 μg/mL, respectively. Moreover, preliminary pharmacokinetic studies revealed that it showed improved oral absorption as compared to the lead compound iodiconazole and deserved for further evaluations.

1. Introduction

The incidence and mortality of invasive fungal infections are rising dramatically due to the increase in the number of immunocompromised or immunosuppressed individuals including patients receiving cancer chemotherapy or organ transplantation, and patients infected with human immunodeficiency virus [1,2]. The three most common species of human fungal pathogens are *Candida albicans* (mortality rate: 20%–40%) [3], *Cryptococcus neoformans* (mortality rate: 20%–70%) [4] and *Aspergillus fumigatus* (mortality rate: 50%–90%) [3]. Clinically, antifungal agents for the treatment of invasive fungal infections include polyenes (*e.g.* amphotericin B) [5], triazoles (*e.g.* fluconazole and itraconazole, see Fig. 1) [6] and candins (*e.g.* caspofungin and micafungin) [7]. Among them, triazoles are the most widely used antifungal agents because of their high therapeutic index, broad spectrum of activity and more favorable safety profile [8]. However, broad application of them also led to severe drug resistance, which has significantly reduced their clinical efficacy. Therefore, it is highly desirable to develop new generation of triazole antifungal agents. Numerous efforts have been made to design and synthesize novel antifungal triazoles and the progress in this field can be found in recent reviews [9–11]. Two of them, isavuconazole and albaconazole (Fig. 1), are candidate new drugs under clinical trials.

Triazole antifungal agents act by competitive inhibition of lanosterol 14α-demethylase (CYP51), a key enzyme in sterol biosynthesis of fungi [12]. In our previous studies, three-dimensional (3D) models of fungal CYP51s were constructed by homology modeling [13–16]. Moreover, highly active triazole analogs were designed and synthesized by our group [15,17–21]. Among them, triazole derivatives shown in Fig. 2 exhibited excellent *in vitro* activity with broad spectrum [17]. However, these triazoles showed low oral bioavailability due to poor water solubility. The heterocycle-benzene bioisosteric replacement is proved to be a useful approach to improve the solubility and pharmacokinetic profile of the lead compound [22–24]. Herein, a series of heterocyclic analogs of the triazoles in Fig. 2 were designed and synthesized. Several target compounds showed excellent antifungal activity with improved oral absorption, which present promising leads for the development of novel antifungal agents.

2. Chemistry

The first step of chemical synthesis is to prepare various chloromethyl heterocycles or bromomethyl heterocycles according to

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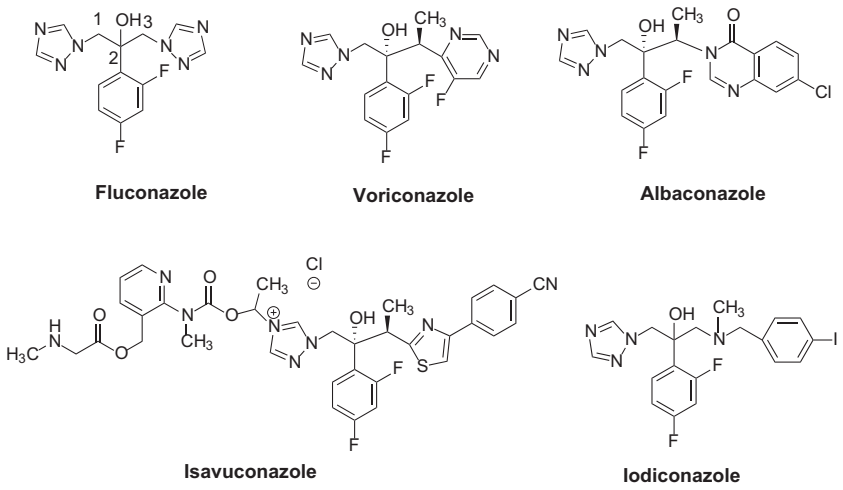

Fig. 1. Chemical structures of representative triazole antifungal agents.

the reported procedures [25,26]. Then, the heterocyclic intermediates were reacted with methylamine or piperazine to give various side chains **5** and **7** (Scheme 1). Finally, the target compounds were obtained as racemates by the ring-open reaction of the oxirane intermediate **4** [18] with intermediates **5** and **7** in the presence of EtOH and a base (Et_3N or K_2CO_3). The isomers of compound **8f** were obtained by chiral HPLC.

3. Microbiology

In vitro antifungal activity was measured according to the National Committee for Clinical Laboratory Standards (NCCLS) recommendations. Serial dilution method in 96-well microtest plate was used to determine the minimum inhibitory concentration (MIC) of the target compounds [18]. Tested fungal strains were obtained from the ATCC or clinical isolates. Briefly, the MIC value was defined as the lowest concentration of tested compounds that resulted in a culture with turbidity less than or equal to 80% inhibition when compared with the growth of the control. Tested 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 *C. neoformans*, and at 7 days for *A. fumigatus*.

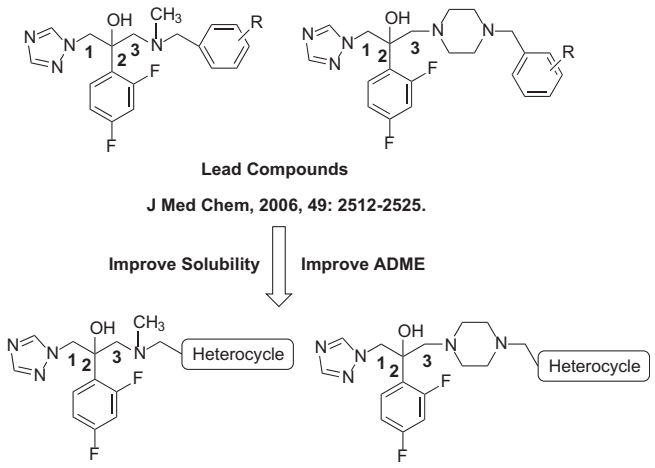

Fig. 2. Design rationale of the target compounds.

4. Results and discussion

4.1. Design rationale

In our previous studies, we reported a series of new azoles with tertiary amine or piperazine side chains (Fig. 2) [17]. These compounds showed good antifungal activity with a broad spectrum. Among them, iodiconazole was developed as a topical antifungal agent for the treatment of dermatomycosis, which is currently under phase III clinical trial [27–29]. Although iodiconazole has excellent *in vitro* antifungal activity, its oral bioavailability was low mainly because of its poor water solubility. Thus, it is highly desirable to improve its oral absorption and develop novel orally active antifungal agent. In the present investigation, the terminal phenyl group of the lead structures was replaced by various heterocyclic groups to afford target compounds **6a**–**e** and **8a**–**f**. This type of bioisosteric replacement was based on the following rationales: (1) the solubility and pharmacokinetic profiles of the compounds should be improved. The heterocycle-benzene exchange is a well-validated approach for the improvement of ADME properties [22]. In particular, this method has been successfully used in the discovery of novel triazole antifungal agents. For example, the replacement of the triazole ring of fluconazole with fluoropyrimidine led to the discovery of voriconazole. Moreover, heterocyclic group seems to be necessary in many marketed or emerging triazole antifungal agents (*e.g.* voriconazole, isavuconazole, albaconazole, see Fig. 1). The calculated LogP values of compounds **6a**–**e** and **8a**–**f** are in the range of 1.92–3.09, suggesting their potential as orally active drugs [30]. (2) Key interactions between the triazole lead and CYP51 should be retained for the bioisosteric replacement. Molecular docking studies revealed that the C3 side chain of the lead compounds (Fig. 2) mainly formed hydrophobic and *van der Waals* interactions with the S4 pocket of *C. albicans* CYP51 (CACYP51) [15]. Analysis of the docking model indicated that five-member or six-member heterocycles and benzoheterocycles can be well accommodated in the active site of CACYP51.

4.2. In vitro antifungal activities

In vitro antifungal activity of the target compounds is reported in Table 1. Fluconazole was used as a reference drug. In general, most of the target compounds showed moderate to good inhibitory