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Design, synthesis, and structure-activity relationship studies of novel tetrazole antifungal agents with potent activity, broad antifungal spectrum and high selectivity

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ABSTRACT

In this letter, we report our efforts to design, synthesize and evaluate biological activities of a series of novel hybridized compounds containing 1-tetrazole and 4-pyridinyl-1,2,4-triazole-3-one. An analysis of structure-activity data indicates that the target compounds with bulky and hydrophobic side chains exhibited stronger activities against the *Candida* spp and *Cryptococcus neoformans* tested than those of fluconazole and racemic VT-1161. Furthermore, **13k** and **13ad** were active against *Microsporum gypseum*, which was resistant to racemic VT-1161. In addition, **13k**, **13ac** and **13ad**, with good *in vitro* activities against all of pathogenic fungi tested except for *Aspergillus fumigatus*, had no inhibition of human CYP3A4, suggesting a low risk of drug-drug interactions.

In recent years, with the appearance of acquired immunodeficiency syndrome, organ transplantation and aggressive treatments of life-threatening cancers, a tremendous growth in the incidence of invasive fungal infections (IFIs) has been inducing increasing morbidity and mortality in patients.[1–3] *Candida albicans*, *Cryptococcus neoformans* and *Aspergillus fumigatus* are the three most common fungal pathogens found in humans.[4] With the use of antifungal agents, however, the incidence of previously less common fungi, such as non-*albicans Candida*, non-*fumigatus Aspergillus*, opportunistic yeast-like fungi and zygomycetes, has increased.[5] Currently, there are three major classes of antifungal agents available to clinicians: polyenes, echinocandins and triazoles. Due to their excellent activity against most of the common fungal pathogens and high therapeutic index, triazole drugs, including fluconazole, voriconazole, itraconazole, posaconazole and isavuconazole (Fig. 1) are widely used.[6]

Triazole antifungal drugs inhibit the activity of fungal 14a-sterol demethylases (CYP51) through reversibly binding the heme iron of CYP51.[7] However, the triazole drugs also inhibit human CYP450s leading to drug-drug interactions (DDIs) and relevant adverse events. For example, the incidence of DDIs is more than 90% among the ICU critical patients receiving mould-active triazole drugs.[8] Even now, DDIs remain problematic.

Based on the above facts, scientists from Viamet Pharmaceuticals speculated that the 1,2,4-triazole of triazole drugs (Fig. 1, red part) possessed strong binding affinity to heme iron, leading to non-selective inhibition of human CYP450s.[9] Thus, they replaced 1,2,4-triazole with 1-tetrazole (Fig. 2, green part) to decrease its affinity for heme iron, and found a highly selective fungal CYP51 inhibitor, VT-1161 (Fig. 2).[9] However, a limited number of fungi were sensitive to VT-1161, which may limit its future applications. Therefore, there is still an urgent need for novel antifungal agents with potent activity and a broad antifungal spectrum.

Molecular hybridization is a useful strategy to discover novel scaffolds with improved affinity and efficacy, and it has been applied widely and successfully to new drug research.[10] In our previous work, we have studied pyridyl-substituted itraconazole analogues (Fig. 2) and obtained an excellent candidate with improved antifungal activities, water solubility and oral bioavailability.[11,12] Herein, we integrated the 1-tetrazole and 4-pyridinyl-1,2,4-tria-

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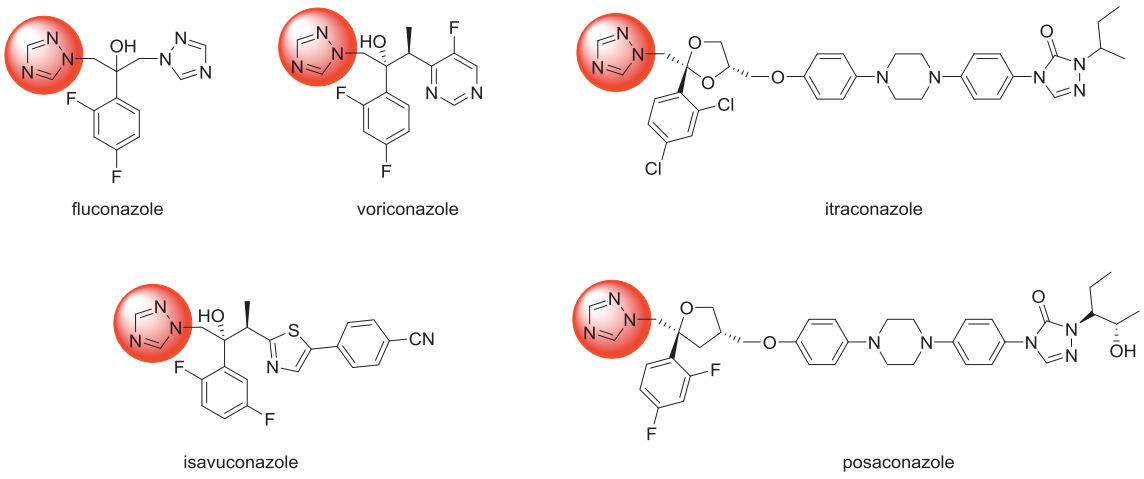

Fig. 1. Triazole drugs for IFIs.

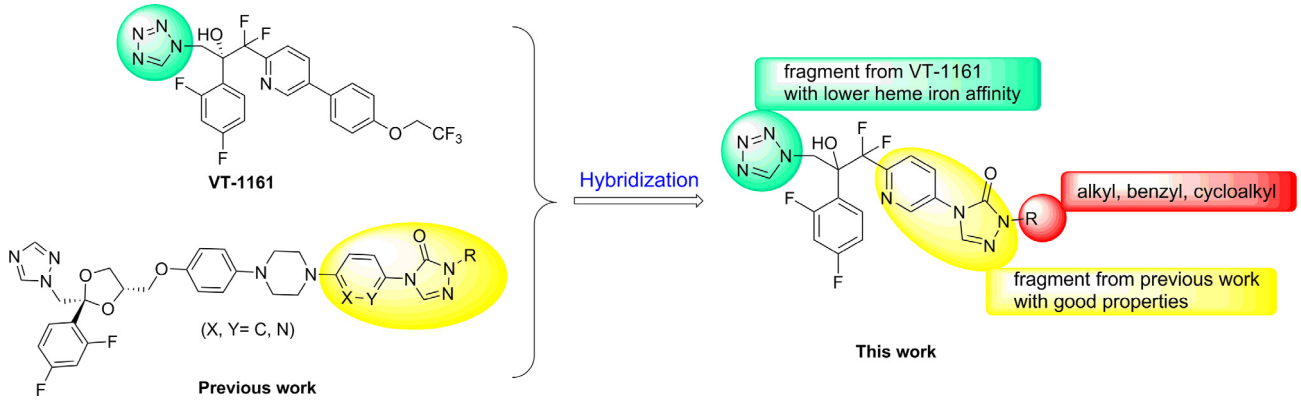

Fig. 2. Design strategy of novel scaffold.

zole-3-one into one molecular platform to generate a novel hybridized scaffold (Fig. 2), and investigated the structure-activity relationship of these hybridized compounds.

The synthetic routes of the target compounds **13a–ao** are depicted in Schemes 1 and 2. First, 2-bromo-5-nitropyridine **1** was reacted with ethyl 2-bromo-2,2-difluoroacetate using Cu and CuI as catalysts to yield compound **2**, which was converted to **3** through hydrolysis. Compound **3** was condensed with N, O-dimethylhydroxylamine to generate amide **4**, which was followed by catalytic hydrogenation to give **5** in good yield. Then, compound **5** was reacted with 4-nitrophenyl carbonochloridate to generate compound **6**. Treatment of **6** with hydrazine hydrate generated compound **7**. Next, intermediate **8** was generated by a microwave-assisted intramolecular ring-closure reaction in the presence of p-toluenesulfonic acid monohydrate. Then, the 1,2,4-triazole-3-one group of **8** was protected with a triphenylmethyl group to yield compound **9**, which was treated with 1-bromo-2,4-difluorobenzene and i-PrMgBr to afford ketone **10**. Compound **10** underwent an epoxidation reaction to yield **11**. Subsequently, the ring-opening reaction of compound **11** with 1H-tetrazole gave compound **12**, which was eventually deprotected to give the key intermediate **13**. The target compounds **13a–ao** were conveniently prepared by nucleophilic substitution reaction between the key intermediate **13** and corresponding alkyl halides or tosylates.

The *in vitro* minimum inhibitory concentrations (MIC) of all target compounds were determined according to the Clinical and Laboratory Standards Institute (CLSI) guidelines.[13,14] The eight pathogenic fungi tested were obtained from the American Type Culture Collection or clinic isolates. Fluconazole purchased from J&K Chemicals and the racemic VT-1161 prepared from our laboratory served as the positive control drugs. The results of *in vitro* antifungal activities are summarized in Tables 1 and 2.

First, we designed and synthesized a series of hydroxyl-substituted and sulfonyl-substituted compounds (Table 1, **13a–f**). The rationale behind this design was that: 1) we have synthesized a series of itraconazole analogues containing hydroxyl-substituted side chains that resulted in improved water solubility and oral bioavailability; and, 2) there is an H-bond interaction between the non-hydroxyl oxygen atom of VT-1161 and His-377 of CYP51 according to the X-ray structure of *C. albicans* CYP51 complexes with VT-1161.[15] We hoped that the hydroxyl-substituted side chains could mimic this H-bond interaction and improve the pharmacokinetic properties. To our surprise, however, all of them were inactive (Table 1). We then calculated the clogP values of the above compounds with ChemBioDraw 14.0 (CambridgeSoft Corporation, America). As shown in Table 1, the clogP values of all the above compounds were less than 1.6, which indicated that the hydrophilic groups were not tolerant. We then synthesized a series of compounds with aliphatic side chains to improve their lipophilicity and evaluated their *in vitro* antifungal activities. To our delight, all target compounds exhibited good antifungal activities against *Candida* spp. and *Cryptococcus neoformans*, which were superior or comparable to the activities of the reference drugs VT-1161 and fluconazole. Notably, **13k** was 2–4-fold more potent than VT-1161 against fungi tested except for *Aspergillus fumigatus*. Furthermore, the antifungal activities of **13g–13k** gradually increased