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Structure-based rational design, synthesis and antifungal activity of oxime-containing azole derivatives

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ABSTRACT

In an attempt to find novel azole antifungal agents with improved activity and broader spectrum, computer modeling was used to design a series of new azoles with piperidin-4-one *O*-substituted oxime side chains. Molecular docking studies revealed that they formed hydrophobic and hydrogen-bonding interactions with lanosterol 14α-demethylase of *Candida albicans* (CACYP51). In vitro antifungal assay indicates that most of the synthesized compounds showed good activity against tested fungal pathogens. In comparison with fluconazole, itraconazole and voriconazole, several compounds (such as **10c**, **10e**, and **10i**) show more potent antifungal activity and broader spectrum, suggesting that they are promising leads for the development of novel antifungal agents.

During the past two decades, there has been a dramatic rise in the incidences of life-threatening systemic fungal infections.[1] This situation can be attributed to the increase in the number of immunocompromised individuals, such as patients undergoing anticancer chemotherapy or organ transplants and patients with AIDS. In these hosts with impaired immune system, the fungal pathogens can easily invade into the tissues and cause serious infections with higher rate of morbidity and mortality.[2,3] *Candida albicans*, *Cryptococcus neoformans*, and *Aspergillus fumigatus* were the most common causes of invasive fungal infections.[4,5] In clinic, antifungal agents that can be used for life-threatening fungal infections are limited. These drugs include amphotericin B[6], 5-fluorocytosine, azoles (such as fluconazole, itraconazole and voriconazole),[7] and echinocandins (such as caspofungin and micafungin).[8] Among them, azoles are the most widely used antifungal agents because of their high therapeutic index. However, the extensive use of azoles has led to the development of severe resistance,[9,10] which greatly reduced their efficacy. Moreover, the clinical efficacy, antifungal spectrum, and DMPK profiles of the azole antifungals are far from satisfactory. This situation has led to an ongoing search for new azoles.[11–16] Several new drugs, such as posaconazole,[17] ravuconazole,[18] and albaconazole[19] are currently in different stages of clinical trials.

Azole antifungals act by competitive inhibition of the lanosterol 14α-demethylase (CYP51), which is the key enzyme in sterol biosynthesis of fungi.[20] As an important target in antifungal chemotherapy, it is of great importance to elucidate the three-dimensional (3D) structures of fungal CYP51s. However, eukaryotic CYP51s are membrane associated proteins and solving their crystal structures remains a challenge. Recently, Podust et al. reported the crystal structure of a prokaryotic sterol 14α-demethylase from *Mycobacterium tuberculosis* (MTCYP51),[21,22] which provided a good template for the modeling of the 3D structures of fungal CYP51s. In our previous studies, we have constructed 3D models of CYP51 from three major fungal pathogens using homology modeling method.[23–25] Important residues involved in azole binding have been investigated by flexible molecular docking[23,24,26] and site-directed mutagenesis.[27] The information obtained from molecular modeling greatly facilitates the process of rational antifungal drug design. Novel non-azole CYP51 inhibitors[28] and highly potent new azoles have been successfully discovered by structure-based rational design.[26]

As a part of our continual effort in azole optimization, we have designed a series of novel azoles with substituted phenoxyalkyl C-3 side chains[29–31] (Fig. 1). Interestingly, various linkers attached to the phenoxyalkyl group played important roles in their antifungal activities. Compounds with N-methyl group and piperazinyl group showed excellent antifungal activity with broad spectrum. When the linker was replaced by the hexahydropyrimidinyl group, the antifungal activity of corresponding compounds was decreased to

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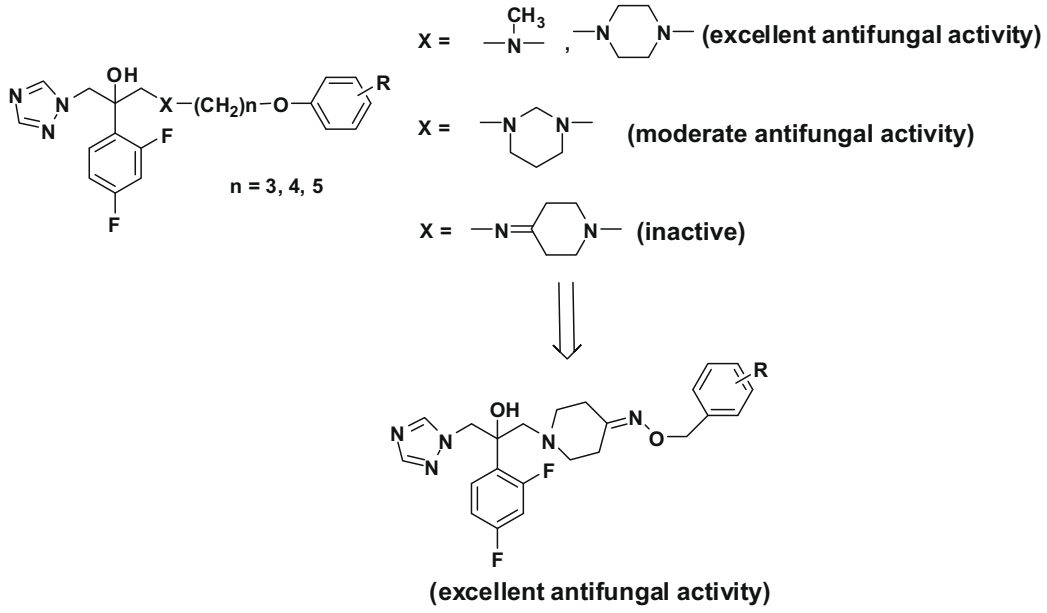

Figure 1. Design rationale of the oxime-containing new azoles.

a large extent. When the linker was changed to the piperidin-4-imino group, the loss of antifungal activity was observed. Molecular docking studies revealed that the hydrogen-bonding interaction between the inhibitor and Ser378 was essential for the antifungal activity. Various linkers could affect the orientation of the side chain and result in different hydrogen-bonding and hydrophobic interactions with CACYP51.

In the present investigation, the inactive piperidin-4-imino compound (Fig. 1) was used as a starting point. Our rationale was focused on modifying the side chain to meet the following three criteria: (1) a side chain that fits well with the active site of CACYP51; (2) a side chain that 'recovers' the hydrogen-bonding interaction with Ser378; (3) a side chain that forms strong hydrophobic interactions with the active site. On the basis of above assumptions, we first reversed the piperidin-4-imino group and replaced the imino group by an oximino group. The oxygen atom of the oxime was supposed to form a hydrogen-bond with Ser378, which is an important residue for the affinity and selectivity of the inhibitors.[26,28–30] Then, various substituted aromatic groups were attached to the oxime oxygen, which can form strong hydrophobic interactions with CACYP51. As a result, a series of new azoles with the piperidin-4-one *O*-substituted benzyl oxime side chains (Fig. 1) were obtained.

In order to validate the hypothesis, compound **10c** was docked into the active site of CACYP51 using the Affinity module within InsightII 2000 software package.[32] Figure 2 showed that compound **10c** shared an extended conformation in the active site, which is similar to that in our reported docking models.[26,29] The oxime side chain was oriented into the S4 pocket[28] and formed hydrophobic and hydrogen-bonding interaction with CACYP51. It is worth noting that the hydrogen-bond between the oxygen atom of the oxime and Ser378 was observed. The terminal benzyl group interacted with surrounding hydrophobic residues such as Phe72, Leu403, and Met374.

The chemical synthesis of the target compounds was outlined in Scheme 1. The key intermediate oxirane **4** was obtained by our reported procedure.[26] The piperidin-4-one *O*-substituted benzyl oxime side chains **9a**–**y** were synthesized via four steps. Piperidin-4-one hydrochloride **5** was treated with excess di-*tert*-butyl dicarbonate to give **6.** Compound **6** was subsequently reacted with excess hydroxylammonium chloride in the presence of sodium hydroxide, ethanol and water at 80 °C to afford **7**. Compound **7** was treated with

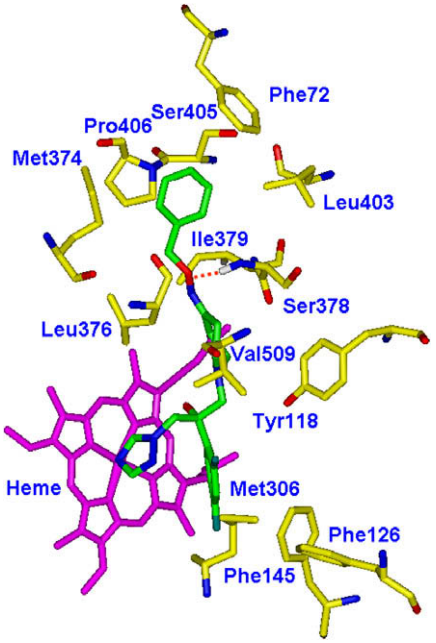

Figure 2. Stereoview of the docking conformation of compound **10c** in the active site of CACYP51. Important residues involved in inhibitor binding are shown and hydrogen-bonds are displayed through red dotted lines.

benzyl bromide in the presence of sodium hydride and DMF to give **8a**–**y**, which were subsequently treated with TFA to afford **9a**–**y**. The target compound **10a**–**y** were obtained as racemates by treating epoxide **4** with compounds **9a**–**y** in the presence of triethylamine and ethanol at 80 °C with moderate to high yields. The compounds **15a** and **15b** (racemates) were obtained using a similar procedure (Scheme 1). The target compound **12** was obtained in two steps. Piperidin-4-one hydrochloride **5** was treated with excess hydroxylammonium chloride in the presence of sodium hydroxide at 80 °C to afford **11**. Compound **12** was obtained as racemates by treating epoxide **4** with **11** in a similar condition.

In vitro antifungal activity of the synthesized azoles was summarized in Table 1. The antifungal activity of each compound was ex-