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Research Letter

Structure-Based Design, Synthesis, and Antifungal Activity of New Triazole Derivatives

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A series of new antifungal triazole derivatives with phenylacetamide side chain were rational designed and synthesized on the basis of the structural information of lanosterol 14-demethylase (CYP51). *In vitro* antifungal activity assay indicated that several compounds showed higher activity than fluconazole. Especially, compound 8h showed excellent inhibitory activity against *Candida albicans* and *Cryptococcus neoformans* (MIC = 0.0156 μg/mL), suggesting that it is a promising lead for the development of novel antifungal agents. The binding mode of compound 8h was investigated by flexible molecular docking. It interacted with CACYP51 through hydrophobic and *van der Waals* interactions.

Key words: antifungal activity, CYP51, phenylacetamide side chain, rational design, triazole

Abbreviations: CYP51, lanosterol 14-demethylase; CACYP51, *Candida albicans* CYP51; AFCYP51, *Aspergillus fumigatus* CYP51; CNCYP51, *Cryptococcus neoformans* CYP51; SAR, structure–activity relationship; MIC, minimal inhibitory concentration; FLZ, fluconazole.

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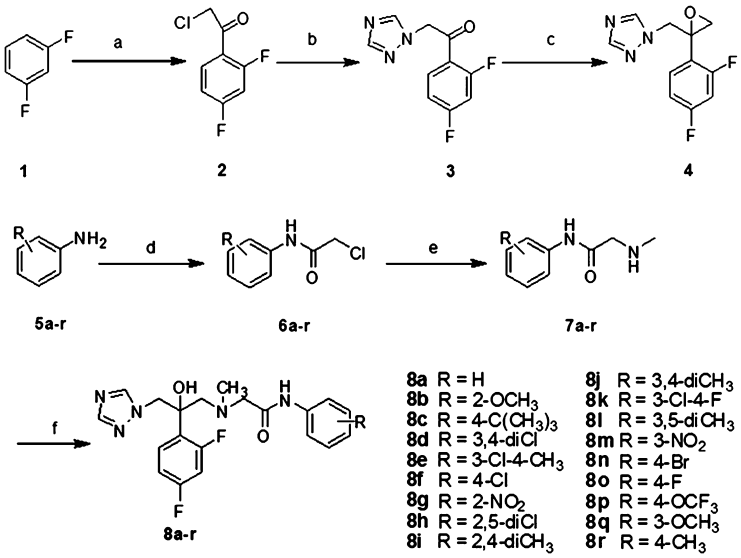
During the past two decades, the incidence of systemic fungal infections has been increasing dramatically because of an increase in the number of immunocompromised hosts (1). In clinic, there are very few antifungal agents that can be used for life-threatening fungal infections. Clinically available antifungal agents include the following: azoles (such as fluconazole, itraconazole, and voriconazole) (2), polyenes (such as amphotericin B and nystatin) (3), echinocandins (such as caspofungin and micafungin) (4), and 5-fluorocytosine. Among those, azoles are widely used as the first-line drug in antifungal therapy. Azole antifungals are competitive inhibitors of the lanosterol 14-demethylase (CYP51), which is the key enzyme in fungal sterol biosynthesis. Eukaryotic CYP51s are membrane-associated proteins, and their crystal structures have not been determined. In our previous studies, we have constructed three-dimensional (3D) models of CYP51 from *Candida albicans* (CACYP51), *Aspergillus fumigatus* (AFCYP51), and *Cryptococcus neoformans* (CNCYP51) through homology modeling (5–7). The binding mode of natural substrate and azoles with CYP51 has been investigated by flexible molecular docking (5,7,8). Important residues involved in azole binding have been validated by site-directed mutagenesis (9).

However, the broad use of azoles has led to the development of severe resistance (10,11), which significantly reduces their efficacy. Moreover, several drawbacks of azoles, such as narrow spectrum, low oral bioavailability, drug–drug interactions, and hepatic toxicity, remain to be overcome. This situation has led to an ongoing search for new azoles (12–17). New triazole antifungal agents, such as ravuconazole (18) and albaconazole (19), are currently in different stages of clinical trials. As a part of our continual effort in azole optimization, azole and non-azole CYP51 inhibitors have been reported (20–23). Herein, structure-based rational drug design was applied to the discovery of a new class of antifungal triazoles with phenylacetamide side chain. The binding mode of the designed compounds was investigated by molecular docking.

The chemical synthesis of the target compounds was outlined in Scheme 1. The oxirane intermediate **4** was obtained by our reported procedure (8). The phenylacetamide side chains **7a-r** were synthesized via two steps. Various substituted anilines were treated with excess 2-chloroacetyl chloride to give compounds **6a-r**, which were subsequently reacted with methylamine in MeOH at room temperature to afford side chains **7a-r.** The target compounds **8a-r** were obtained as racemates by treating epoxide **4** with side chains **7a-r** in the presence of triethylamine and EtOH at 80 °C (Appendix S1).

Our previous molecular modeling studies indicated that the active site of CACYP51 can be divided into four pockets (24). The triazole ring, difluorophenyl group, and C2-hydroxyl group are common skeleton of triazole antifungal agents. Our docking studies revealed that the triazole ring bound to the S2 pocket through the formation of a coordination bond with iron of heme group. The C-2 hydroxyl group was supposed to interact with the S1 pocket through the hydrogen-bonding interaction with His310. The S3 pocket represents the narrow and hydrophobic cleft (facing BC loop) and formed hydrophobic interaction with the difluorophenyl group. Most of the recent efforts for azole optimization have been focused on the design of various



Scheme 1: Reagents and conditions: a. $ClCH_2COCl$, $AlCl_3$, CH_2Cl_2, 40 °C, 3 h, 50%; b. $C_2H_3N_3$, K_2CO_3, CH_2Cl_2, rt, 24 h, 70.0%; c. $(CH_3)_3SOI$, NaOH, toluene, 60 °C, 3 h, 62.3%; d. $ClCH_2COCl$, CH_2Cl_2, $(CH_3CH_2)_3N$, 0 °C, 4 h, 50.2–75.3%; e. CH_3NH_2, K_2CO_3, methanol, rt, 24 h, 95.0–98.9%; f. **4**, $(CH_3CH_2)_3N$, ethanol, reflux, 9 h, 18.3–30.5%.

C3 side chains (8,12,25,26), which were found to be located in the S4 pocket. The S4 pocket represents a hydrophobic hydrogen bond-binding site (facing FG loop) and can bind with chemically diverse side chains. The structure–activity relationship (SAR) of C3 side chains remains to be further explored. According to the hydrophobic nature of the S4 pocket, we designed 2-(methylamino)-*N*-phenylacetamides as side chains to form good hydrophobic and *van der Waals* interactions with it (Figure 1). To validate the binding mode of designed azoles, compound **8h** was docked into the active site of CACYP51 by the affinity module within InsightII software package.[a] The docking protocols were similar to those in our previous studies (20). Figure 2 revealed that the phenylacetamide side chain of compound **8h** was oriented into the S4 pocket with an extended conformation. The *N*-methyl group formed hydrophobic and *van der Waals* interactions with Val509 and Phe228. The amide group did not form direct interaction with the active site, but it was important for the orientation of the side chain. The substituted terminal phenyl group interacted with surrounding hydrophobic residues such as Leu403, Val404, Leu376, and Leu461.

In vitro antifungal activity of the synthesized compounds is shown in Table 1. The antifungal activity of each compound was depicted as the minimal inhibitory concentration (MIC) that achieved 80% inhibition of the tested pathogenic fungi with FLZ used as a reference drug. In general, all the synthesized compounds show moderate-to-excellent activity against all the tested fungal pathogens

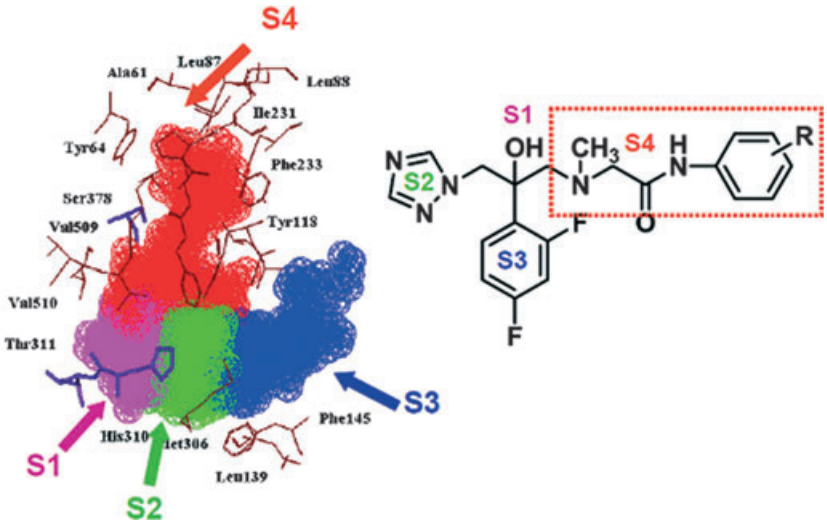


Figure 1: Design rationale of the phenylacetamide-containing new azoles.

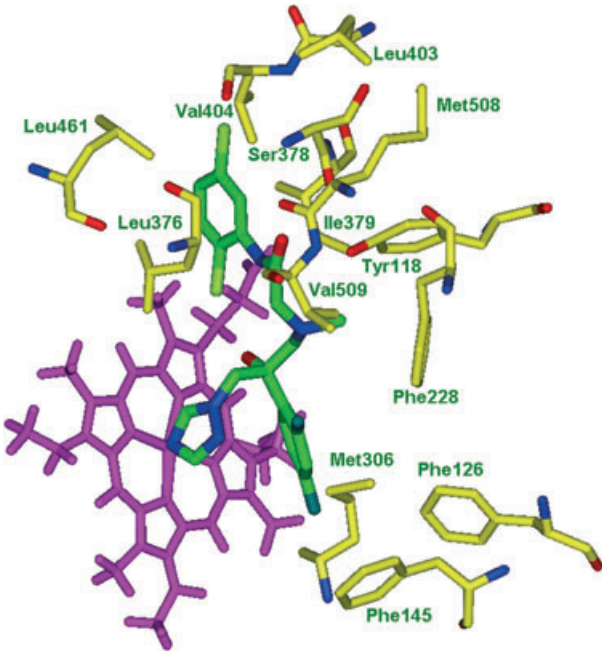


Figure 2: The docking conformation of compound **8h** in the active site of CACYP51. Important residues involved in inhibitor binding are shown.

except compound **8m**. The target compounds revealed the highest activity against *C. albicans* and *C. neoformans*. *C. alibicans* has a worldwide distribution and is the most common cause of life-threatening fungal infections. The MIC_{80} value of compounds **8b**, **8c**, **8d**, **8e**, **8f**, and **8p** against *C. albicans* is 0.25 μg/mL, indicating that their activity is comparable with that of FLZ. Especially, compound **8h** is almost 16-fold more potent than FLZ. Moreover, several compounds (e.g., **8c**, **8d**, and **8e**) also showed comparable or superior inhibitory activity against other *Candida* spp. (such as *C. tropicalis* and *C. parapsilosis*) to that of FLZ. Most of the compounds only show moderate activity against *C. krusei*. Only compound **8h** (MIC = 0.0625 μg/mL) shows better activity than FLZ. *C. neoformans* is another major cause for life-threatening fungal infections. For most of the synthesized compounds, their MIC values are in the range of 0.016–1 μg/mL. Among them, the activity of compound **8c** (MIC = 0.0625 μg/mL) is comparable with that of FLZ, while compound **8h** (MIC = 0.0156 μg/mL) shows higher activity than FLZ. Compound **8h** exhibits the highest activity with broad antifungal spectrum, which is worthy of further evaluation.

Preliminary SARs of the synthesized azoles were summarized from their antifungal activity data. The terminal phenyl group was found to be located in a hydrophobic pocket lined with Leu403, Val404, Leu376, and Leu461. Hydrophobic substitutions at proper position of the phenyl ring are helpful to improve the binding affinity with CACYP51. On the contrary, substitutions at unreasonable position might lead to the steric clashes with CACYP51. Therefore, the substitutions on the terminal phenyl group play an important role for the antifungal activities. In general, most of the 4-substituted derivatives show higher antifungal activity than 2-substituted and 3-substituted derivatives except compound **8b**. For the latter, methoxyl group (e.g., compounds **8b** and **8q**) is more favorable for the antifungal activity than nitro group (e.g., compounds **8g** and **8m**). 2-Methoxyl derivative **8b** showed comparable activity with the 4-substituted compounds. For the substitutions on position 4, *tert*-butyl group, chlorine, and trifluoromethoxyl group are favorable for the antifungal activity. Compared with compound **8a**, the introduction of fluorine (compound **8o**) or bromine (compound **8n**) on the position 4 of the phenyl group has little effect on the antifungal activity. On the other hand, the introduction of a methyl group (compound **8r**) led to the decrease in the antifungal activity. Moreover, several di-substituted derivatives show improved antifungal activity. For example, the 2,5-dichloro derivative **8h** is the most active compound. 3,4-Dichloro derivative **8d** also shows good antifungal activity. For the di-methyl-substituted compounds (**8i**, **8j**, and **8l**), they

Table 1: Antifungal *in vitro* activities of the compounds (MIC_{80}, $\mu g/mL^1$)

Compounds	R	*Candida albicans*	*Candida tropicalis*	*Candida parapsilosis*	*Candida krusei*	*Candida neoformans*
8a	H	1	16	16	16	1
8b	2-OCH_3	0.25	1	1	4	1
8c	4-$C(CH_3)_3$	0.25	0.0625	0.25	1	0.0625
8d	3,4-diCl	0.25	0.25	0.25	1	0.25
8e	3-Cl-4-CH_3	0.25	0.25	0.25	1	0.25
8f	4-Cl	0.25	1	1	4	0.25
8g	2-NO_2	4	4	16	4	4
8h	2,5-diCl	0.0156	1	1	0.0625	0.0156
8i	2,4-diCH_3	1	64	16	4	1
8j	3,4-diCH_3	1	4	4	4	1
8k	3-Cl-4-F	1	1	4	4	1
8l	3,5-diCH_3	1	4	4	16	1
8m	3-NO_2	>64	>64	>64	>64	64
8n	4-Br	1	1	4	16	1
8o	4-F	1	64	1	64	1
8p	4-OCF_3	0.25	16	0.25	4	0.25
8q	3-OCH_3	4	16	4	64	1
8r	4-CH_3	16	64	1	64	4
FLZ		0.25	0.25	0.25	1	0.0625

showed the same activity against *C. albicans* and *C. neoformans* as the unsubstituted derivative **8a**. Interestingly, 3,4-dimentyl derivative **8j** and 3,5-dimentyl derivative **8l** are more active against *C. tropicalis* and *C. parapsilosis* than compound **8a**.

In summary, structure-based drug design was used to discover new antifungal azoles with phenylacetamide side chain. *In vitro* antifungal activity assay indicated that the new azoles showed moderate-to-good activity against invasive fungal pathogens. Compound **8h** showed the best inhibitory against *C. albicans* and *C. neoformans* (MIC = 0.0156 μg/mL). Molecular docking studies revealed that it interacted with CACYP51 mainly through hydrophobic and *van der Waals* interactions. Because the phenylacetamide represents a new type of side chain in azole antifungal agents, further structural modification is essential to obtain more information about SAR.

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Note

[a]InsightII 2000, Molecular Simulation Inc., CA, USA, 1999.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Experimental details, NMR, and MS data for the synthesized compounds.

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