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New triazole derivatives as antifungal agents: Synthesis via click reaction, in vitro evaluation and molecular docking studies

Yan Zou [a,†], Qingjie Zhao [a,†], Jun Liao [a], Honggang Hu [a], Shichong Yu [a], Xiaoyun Chai [a], Mingjuan Xu [b,*], Qiuye Wu [a,*]

[a] Department of Organic Chemistry, College of Pharmacy, Second Military Medical University, Guohe Road 325, Shanghai 200433, People's Republic of China
[b] Department of Gynecology and Obstetrics, Changhai Hospital, Second Military Medical University, Shanghai 200433, People's Republic of China

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ABSTRACT

A series of 1-(1*H*-1,2,4-triazol-1-yl)-2-(2,4-difluorophenyl)-3-substituted-2-propanols (**5a**–**5y**) which are analogues of fluconazole, have been designed and synthesized via Cu(I)-catalyzed azide–alkyne cycloaddition on the basis of computational docking experiments to the active site of the cytochrome P450 14α-demethylase (CYP51). The in vitro antifungal activities of all the target compounds were evaluated against eight human pathogenic fungi. Compound **5l** showed the best antifungal activities.

Fungal infections pose a continuous and serious threat to human health and life especially to immunocompromised patients.[1–3] Clinically, candidosis, aspergillosis and cryptococcosis are three major fungal infections in immunocompromized patients.[4,5] Currently, triazole agents (fluconazole, itraconazole, voriconazole and posaconazole, Fig. 1) are the most frequently used antifungals in the clinic.[6] However, fluconazole is not effective against invasive aspergillosis and has suffered severe drug resistance.[7,8] This situation highlights the need for new triazole derivatives possessing broader antifungal spectra and higher therapeutic indexes.

Azole antifungals act by competitive inhibition of CYP51, the enzyme that catalyzes the oxidative removal of the 14a-methyl group of lanosterol to give $\Delta^{14,15}$-desaturated intermediates in ergosterol biosynthesis.[9] In general, the active site of CYP51 for ligand binding can be divided into three subsites: the hydrophilic H-bonding region, the hydrophobic region, and the narrow hydrophobic cleft formed by the residues in the helix β′-meander 1 loop and N-terminus of helix I.[10]

Some studies[11–14] have revealed a pharmacophore of antifungal triazoles, which contains a triazole ring linked to a dihalophenyl ring through a two carbon chain. In addition, the carbon alpha to the phenyl ring bears a hydroxyl group. But the side chains located in the narrow hydrophobic cleft were also very important. We intended to alter the side chains to find potent systemic antifungal compounds with a broad antifungal spectrum and less potential to develop resistance.

According to the above results, we designed a series of 1-(1*H*-1,2,4-triazole-1-yl)-2-(2,4-difluorophenyl)-3-substituted-2-propanols (**5**, Fig. 2) containing a triazole ring, a difluorophenyl group, a hydroxyl group and a side chain. In our design, we systematically altered the structure of fluconazole as a platform and tried to insert 1,2,3-triazolyl group into the side chain via Cu(I)-catalyzed azide–alkyne cycloaddition.

Compounds **5a–5y** were synthesized according to a very efficient and straightforward synthetic route outlined in Scheme 1. After the key intermediate **3** was synthesized by a known procedure,[15] Compound **4** was synthesized by Nucleophilic addition reaction of **3** with propargyl bromide in the presence of Zn in DMF at room temperature.[16] The click reaction approach toward the synthesis of the novel 1,2,3-triazolyl linked triazole antifungal derivatives **5a–5z** was achieved by Cu(I)-catalyzed 1,3-dipolar cycloaddition with substituted azidomethyl benzene.[17] All the new compounds described above were characterized by ^{1}H NMR, ESI-MS and HR-MS spectroscopic analysis.[18]

The in vitro minimal inhibitory concentrations (MICs) of the compounds were determined by the micro-broth dilution method in 96-well microtestplates according to the methods defined by the National Committee for Clinical Laboratory Standards (NCCLS).[19] The in vitro antifungal activities of all the target compounds were

* Corresponding authors. Tel./fax: +86 21 8187 3592 (M.X.); tel./fax: +86 21 8187 1225 (Q.W.).
E-mail addresses: mjxu68@gmail.com (M. Xu), smmuwuqy@gmail.com (Q. Wu).
† These two authors contributed equally to this work.

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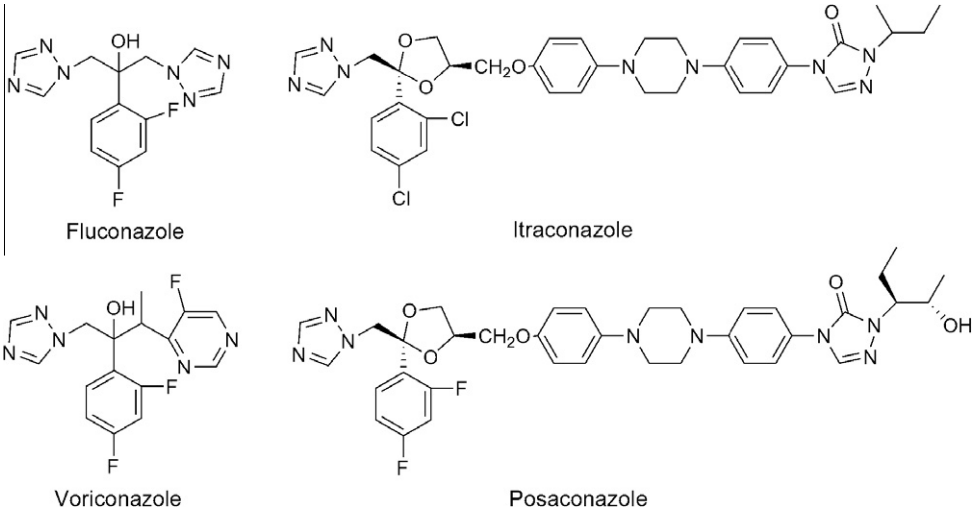
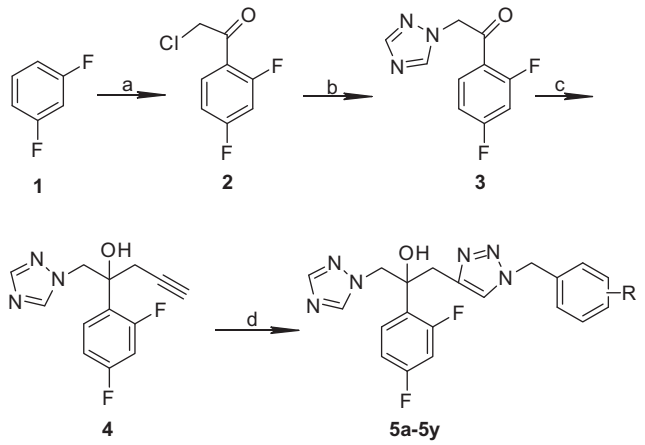


Figure 1. Triazole antifungal agents used in clinical therapy.



Scheme 1. Synthetic routes of the target compounds. Reagents and conditions: (a) $ClCH_2COCl$, $AlCl_3$, 50 °C, 5 h, 82%; (b) 1*H*-1,2,4-triazole, $NaHCO_3$, toluene, reflux, 5 h, 62%; (c) Zn, propargyl bromide, DMF/THF, 60 °C, 6 h; 95%; (d) NaN_3, substituted benzyl bromide, ascorbate sodium, $CuSO_4$, DMSO, 84%.

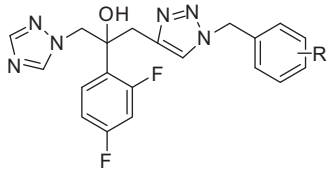


Figure 2. General structure of the designed fluconazole analogues.

evaluated against eight human pathogenic fungi (*C. alb.SC5314*, *C. alb.Y0109, C. neo.*, *C. par.*, *C. tro., T. rub., C. kef., A. fum.*) which are often encountered clinically and are summarized in Table 1. The MIC values (in μg/mL) against different pathogenic fungi, in comparison with itraconazole (ICZ), ketoconazole (KCZ), amphotericin B (AMB), voriconazole (VCZ) and fluconazole (FCZ).

The results of antifungal activities in vitro showed that all of the 25 target compounds **(5a–5y)** were active against nearly all fungi tested to some extent (Table 1). Most of the target compounds exhibited higher activities against *C. alb Y0109*, *C. alb SC5314* and *C. kef* than all five positive controls. Obviously, the antifungal activity of compounds (**5j–5l, 5x–5y**) was better than that of the other compounds. The MIC_{80} value of compound **5l** is 128 times lower than that of FCZ against *C. alb. SC5314* in vitro (with the MIC_{80} value of 0.0039 μg/mL), and 256 times lower than that of FCZ against *C. kef.* (with the MIC_{80} value of 0.0156 μg/mL). The MIC_{80} value of compound **5u** is 16 times lower than that of FCZ against *C. tro* and 64 times lower than that of FCZ against *C. kef* in vitro (with the MIC_{80} value of 0.0625 μg/mL).Compounds **5l, 5t, 5y** and **5z** are worthy of further study, and are expected to be developed into new antifungal drugs.

To explain the results, we proposed a hypothetical binding mode for **5l** to the active site of CYP51 based on computational docking results (Fig. 3). As usual, the triazole interacts with iron of the heme group, while the 2,4-difluorophenyl group in the designed compound could be placed into the hydrophobic pocket formed by Gly114, Phe126, Leu139, Met140, Phe145, Ile304 and Met306. The side chain incorporate to adjust the overall physical-chemical properties of the molecules and orientation of aromatic rings. It would also be oriented to interact with a hydrophobic pocket formed by Leu121, Thr122, Phe228, Thr311, Pro375, Leu376, His377, Ser378, Met508, Val509 and Val510. The 1,2,3-triazole group in the side chain would generate π–π stacking interactions with the Tyr118. Finally, the substituted benzyl could interact with a hydrophobic pocket formed by Ala114, Phe126, Gln142 and Phe145, it could also generate π–π stacking interactions with Phe380.

In addition, the side chains were the pharmacophores, and the spatial orientations of the pharmacophores were just oriented in the hydrophobic pocket. The side chains were very important. They played a role in adjusting the physical-chemical properties of the whole molecule to avoid some negative side effects and improved their pharmacokinetic and pharmacodynamic behavior.

In conclusion, an efficient method using the click reaction has been developed for the synthesis of diversified novel triazole derivatives. Results of preliminary antifungal tests against eight human pathogenic fungi in vitro showed that these analogs exhibited excellent activities with a broad spectrum. The obtained results indicated that for antifungal activity of these novel triazole derivatives it is very helpful to introduce the 1,2,3-triazolyl group and the substituted benzyl groups as side chains. This research has led to the discovery of a series of compounds for further optimization.