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Synthesis and biological evaluation of vinyl ether-containing azole derivatives as inhibitors of *Trichophyton rubrum*

Lulu Wang [a], Wenge Yang [b], Kai Wang [a], Jing Zhu [b], Fei Shen [c], Yonghong Hu [a,*]

[a] College of Biotechnology and Pharmaceutical Engineering, Nanjing University of Technology, Nanjing 210009, China
[b] School of Pharmaceutical Sciences, Nanjing University of Technology, Nanjing 210009, China
[c] Jiangsu Engineering Technology Research Center of Polypeptide Pharmaceutical, Nanjing 210009, China

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ABSTRACT

In an attempt to search for many target compounds with excellent activities, a series of vinyl ether-containing azole derivatives were designed, synthesized, and evaluated as antifungal agents. Results of preliminary antifungal tests against *Trichophyton rubrum* in vitro indicated that most of the synthesized compounds showed excellent activities. In comparison with fluconazole, itraconazole, voriconazole, omoconazole and amphotericin B, several compounds (such as **7d**, **7g** and **7h**) exhibited more potent inhibitory activities, suggesting that they were promising leads for the development of novel antifungal agents.

During the past several decades, fungal infections have become a continuous and serious threat to human health and life particularly in immunocompromised individuals.[1–3] Fungal infections include superficial and systemic fungal infections. The anthropophilic species *Trichophyton rubrum* is an important etiologic agent of tinea (ringworm) infections, including onychomycosis (nail infection), tinea cruris (groin infection), and tinea pedis ('athlete's foot').[4] It is reported that about 90% of the chronic dermatophyte infections are caused by *T. rubrum*. These conditions are relatively benign and easy to treat, except in immunocompromised patients, but therapy is costly and can fail in a significant proportion of cases.[5]

Currently, numerous antifungal drugs such as amphotericin B, 5-fluorocytosine, azoles, and echinocandins (including caspofungin and micafungin) spring up.[6] However, because of the emergence of drug resistance, undesirable side effects, high risk of toxicity and insufficiencies in their antifungal activity, the clinical uses of most agents have been limited. For this reason, it is necessary to develop and extend the safe and efficient chemotherapeutic agents with potent antifungal activities.[7]

Among these antifungal drugs, azoles (such as fluconazole, itraconazole and voriconazole) are the most widely used antifungal agents due to their high therapeutic index. Azole antifungals act by inhibiting the biosynthesis of ergosterol, the bulk sterol in fungal membranes, by the heterocyclic nitrogen atom (N-3 of imidazole, N-4 of triazole) binding to the heme iron atom located in the active site of the cytochrome P450 14α-demethylase (CYP51), which may disrupt the close packing of acyl chains of phospholipids, impairing the functions of certain membrane bound enzymes, such as ATPase and enzymes of the electron transport system. Together, these actions inhibit fungal growth.[8–10] However, the extensive use of such antifungal agents has led to the development of severe resistance, which significantly reduced their efficacy.[11,12] Survey reveals some clinically used drugs, such as fluconazole, may also cause resistance to new structurally related azoles, for example, voriconazole and ravuconazole.[13–15] Hence, the discovery of novel and potent antifungal azoles is very important and necessary to overcome this situation and develop effective therapies.

Up to the present, the structurally and functionally important regions have been recognized accurately. In general, the active site of CYP51 for ligand binding can be divided into four subsites: a coordination bond with iron of the heme group, the hydrophilic H-bonding region, the hydrophobic region, and the narrow hydrophobic cleft formed by the residues in the helix B'-meander 1 loop and N-terminus of helix I.[16] Important residues involved in azole binding have been investigated by flexible molecular docking[17–19] and site-directed mutagenesis.[20] The molecular modeling that gives the utilization of structural information of fungal CYP51s greatly facilitates the process of rational antifungal agents design.

Omoconazole (Fig. 1) which developed by Siegfrieds's research laboratories is an imidazole antifungal and has been widely used

* Corresponding author. Tel./fax: +86 25 83587108.
E-mail address: yonghonghu09@gmail.com (Y. Hu).

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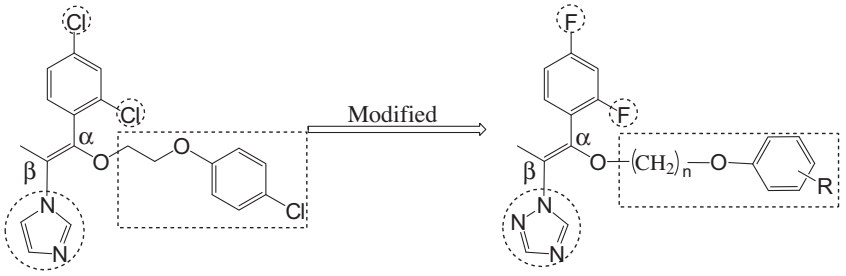
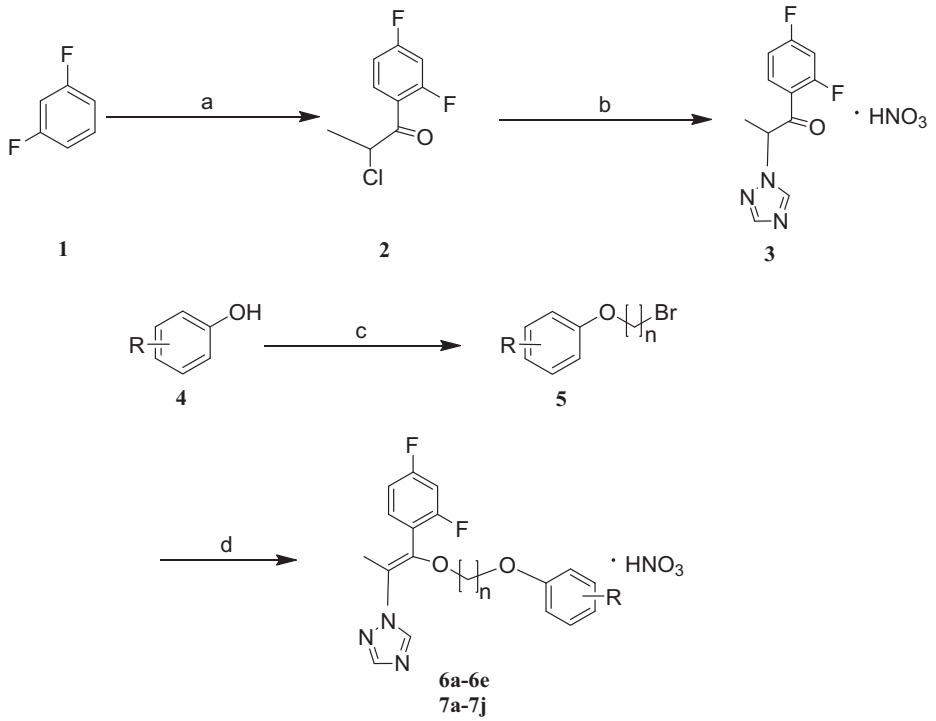


Figure 1. Chemical structure of omoconazole and modified positions of the compounds.

for the treatment of superficial fungal infections, including dermatophytoses, pityriasis versicolor, molds, and cutaneous and vaginal candidiasis. It also has bacteriostatic effect on Gram-positive bacteria such as Staphylococcus aureus and Group A and D Streptococci.[10] The spectrum of antifungal activity of omoconazole has been reported to be quite similar to that of the imidazole family, such as tioconazole and econazole. A number of clinical studies show that omoconazole has exhibited excellent tolerance and highly effective in the treatment of vaginal candidiasis as vaginal ovules, in the treatment of dermatomycoses and tinea versicolor as a 1% cream formulation and spray, and in the treatment of candidal balanitis as a 1% spray powder formulation.[21] Researches indicated that the imidazole ring, the dichlophenyl group and the vinyl ethers were the pharmacophores of omoconazole. Furthermore, it is reported that the vinyl ethers structures were very important and helpful for optimal antifungal activity, stability and toxicological properties. In addition, introduction of a double bond between α and β carbon atoms leads to stereoisomers differing in physicochemical and biological parameters and increasing the spatial demand at the α carbon by alkylation improved the desired properties.[22]

In our compounds design, we systematically altered the structure of omoconazole using the strategy of structure-based rational drug design. The modification was focused mainly on the side chains at the α-position of 1-(1*H*-imidazolyl)methylarylketones. The side chains which located in the narrow hydrophobic cleft were also important.[16] And the optimization of the side chain attached to the pharmacophore is attractive to the current researches. Besides, some researchers suggested that triazole antifungals, such as fluconazole, voriconazole and posaconazole, were more efficient than imidazole antifungals and the activity of a drug with the diflurophenyl group at the pharmacophore was higher than that with the dichlophenyl group.[23,24] To sum up, we intended to find highly potent new antifunal drugs with excellent antifungal activity, low toxicity and less potential to develop resistance.

The general synthetic methodology for the preparation of the target compounds **6a–6e** and **7a–7j** was accomplished using chemistry illustrated in Scheme 1. As a key intermediate of our designed vinyl ether-containing azole antifungals, the compound **3** was synthesized with known procedures.[19] Compound **5** were another key intermediates and they can normally be prepared from alkylation



Scheme 1. Synthesis route to the title compounds. Reagents and conditions: (a) $CH_3CHClCOCl$, $AlCl_3$, 55 °C, in 91% yield; (b) 1*H*-1,2,4-triazole, K_2CO_3, CH_2Cl_2, rt, 24 h, HNO_3, 55%; (c) 1,*n*-dibromoalkane, NaOH, tetrabutyl ammonium bromide(TBAB), DMF, reflux, 8 h, 66–80%; (d) **3**, NaOH, tetrabutylammonium hydroxide(10% in water), toluene, 50 °C, 20 h, 65% HNO_3, 40–62%.[27,28]

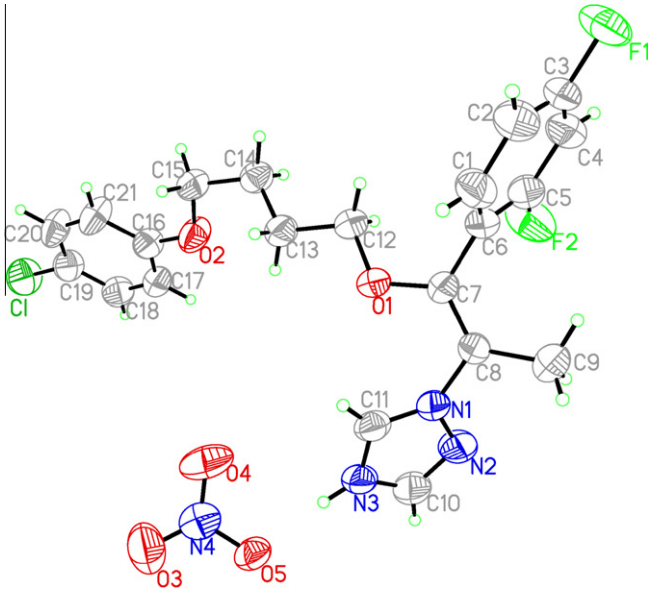


Figure 2. X-ray analysis of compound **6c**.

Table 1
Antifungal activities of the title compounds in vitro (MIC_{80}, μg/mL)[a]

Compound	*n*	*R*	*T. rubrum*
6a	2	4-Cl	0.2
6b	3	4-Cl	0.125
6c	4	4-Cl	**0.0625**
6d	5	4-Cl	0.25
6e	6	4-Cl	2.5
7a	3	4-Br	0.25
7b	3	4-F	0.1
7c	4	2-Cl	0.1
7d	4	3-Cl	**0.025**
7e	4	2,4-Cl	2
7f	4	2,4,6-Cl	>20
7g	4	4-Br	**0.05**
7h	4	4-F	**0.025**
7i	3	$4\text{-}CH_3$	2.5
7j	4	$4\text{-}CH_3$	0.2
FCZ			4
ICZ			0.125
VCZ			0.0625
OCZ			0.1
AMB			2

[a] *Abbreviations: T. rubrum*, *Trichophyton rubrum*; FCZ, fluconazole; ICZ, itraconazole; VCZ, voriconazole; OCZ, omoconazole; AMB, amphotericin B.

of substituted phenols **4** by using 1,*n*-dibromoalkanes in the presence of NaOH, catalytic amount of tetrabutyl ammonium bromide (TBAB) in DMF. To a stirred mixture of **3** and **5** in the presence of 50% aqueous soda lye, tetrabutylammonium hydroxide(10% in water) as phase transfer catalyst in toluene and then mixed with 65% aqueous nitric acid, we can afford the compounds **6a–6e**. The target compounds **7a–7j** were synthesized in the same way as **6a–6e**. All the new compounds described above were characterized by LC–MS, and NMR spectroscopic analysis.[25] Moreover, we did X-ray analysis of some compounds, for example, compound **6c** (Fig. 2).[26]

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).[29] The MIC_{80} was defined as the first well with an approximate 80% reduction in growth compared to the growth of the drug free well. For assays, the title compounds to be tested were dissolved in dimethyl sulfoxide (DMSO), serially diluted in growth medium, inoculated and incubated at 35 °C. Growth MIC was determined at 72 h for *T. rubrum*. Fluconazole(FCZ), itraconazole(ICZ), voriconazole (VCZ), omoconazole(OCZ), and amphotericin B(AMB) were obtained from their respective manufacturers served as the positive control. The results of assays were summarized in Table 1. The data were obtained from the mean of replicates. All of our susceptibility tests were performed three times.

In vitro antifungal activity assay (Table 1) indicates that most of the synthesized compounds (**6a–6e**, **7a–7j**) show moderate to excellent activity against *T. rubrum*. From the MIC_{80} values of **6a–6e** series, we can see that compounds **6b** ($n = 3$) and **6c** ($n = 4$) show higher antifungal activities than those of the others. Especially, the activity of compound **6c** is comparable to that of voriconazole and superior to that of omoconazole and itraconazole. So we choose $n = 3,4$ for the synthesis of the **7a–7j** series, particularly $n = 4$. Noticeably, compounds **7d**, **7g** and **7h** show higher inhibitory activities than those of all the positive controls. The MIC_{80} values of compounds **7d** and **7h** are 160 times lower than that of fluconazole against *T. rubrum* in vitro, indicating that they are promising leads for the discovery of novel antifungal agents.

From the antifungal activity data, preliminary structure-activity relationships (SARs) of the synthesized compounds were obtained. First of all, we substituted the triazole ring and the diflurophenyl group for imidazole ring and dichlophenyl group at the pharmacophore respectively (compound **6a**). The MIC values of compound **6a** and omoconazole were almost the same, which indicated that these modifications could not improve the inhibitory activity against *T. rubrum* effectively. We noticed that the side chains at the α-position of 1-(1*H*-triazolyl)methylarylketones which located in the narrow hydrophobic cleft of CYP51 were very important to the activity. So the modification was focused mainly on the side chains. We wanted to find out how chain length and the substitutions on the phenyl ring could influence the inhibitory activity. Having a number of reactive reagents compound **5**, we tried these on compound **3**, which led us to obtain the target compounds (**6a–6e**,**7a–7j**) containing vinyl ether structure. Compared with omoconazole, increasing the spatial demand at the α carbon by alkylation could improve the desired properties. From the MIC_{80} values of **6a–6e** series, we could find that the alkyl of 4 carbon atoms (compound **6c**) exhibited higher inhibitory activities than that of the other lengths of carbon chain. In addition, the substitutions on the phenyl ring which could form strong hydrophobic interactions with the active site of CYP51 played an important role for the antifungal activities. In general, halogens group (e.g. compounds **6b**, **6c**, **7d**, **7g** and **7h**) were favorable for the antifungal activity. On the contrary, the introduction of methyl (compounds **7i** and **7j**) did not show improved antifungal activity, which might indicate that the alkyl groups on the phenyl ring could not effectively improve the inhibitory activity in this study. For compounds **6c**, **7c** and **7d**, the position of the substitutions also had influence on the antifungal activity. The phenyl group substituted by chlorine in the 3 position (compound **7d**) showed higher inhibitory activity against *T. rubrum* than that in the 4 or 2 position. Besides, from the MIC_{80} values of **6c**, **7g** and **7h**, we could obtain that the phenyl substituted by fluorine showed better antifungal activity than that by chlorine or bromine in the same position. In comparison with the mono-chlorine substituted compounds (**6c**, **7c** and **7d**), the di- and tri-substituted derivatives (**7e** and **7f**) led to the decrease of the antifungal activity to a large extent. Because it is a new type of side chain in azole antifungal agents, futher structural modification is essential to obtain more information about SARs.

In conclusion, a new class of vinyl ether-containing azole derivatives had been synthesised. Results of preliminary antifungal test against *T. rubrum* in vitro showed that these analogs exhibited

excellent activities. The vitro antifungal assay also indicated that a proper length of side chain and the substitutions on the phenyl ring played very important roles for the antifungal activities. Several compounds, such as **7d** and **7h**, were promising leads for the discovery of novel antifungal agents. Effort aimed at further optimization, as well as in-depth biological investigations, of the identified lead compounds is continuing in our laboratories, and results will be reported in due course.

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25. Experimental: *Representative analytical data for compound **6a***. A mixture of 1-(2,4-difluorophenyl)-2 -(1,2,4-triazol)-1-yl)propan-1-one (3.0 g, 0.01 mol), 10 g of a 50% aqueous soda lye and 1.5 mL tetrabutylammonium hydroxide as phase transfer catalyst in toluene (15 mL) was stirred and heated to 50 °C. Then 1-bromo-2-(4-chlorophenoxy)-ethane (2.4 g, 0.01 mol) dissolved in 10 mL toluene, was instilled into the reaction solution. The mixture was subsequently stirred for another 20 h at 50 °C (monitored by TLC, eluent, ethyl acetate/petroleum ether, 1/1, v/v). Then the solvents were evaporated under reduced pressure, and the residue was treated with water (100 mL) and extracted with chloroform (3 × 100 mL). The organic layers were combined, dried with anhydrous Na_2SO_4, and concentrated under reduced pressure. The remaining residue was a dark oil that was diluted with 10 ml 2-propanol and then adjusted to a pH-value of 2 by 65% HNO_3. The thus derived nitric acid solution was then cooled in the refrigerator. The impure precipitated product herein was subsequently crystallized from the mixture solvent of ethyl acetate/ethanol (1:1, v/v) to afford compound **6a** (2.8 g) as white solid. Yield: 62%; mp: 127–129 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 9.71 (1H, s, TriazC_3-H), 8.31 (1H, s, TriazC_5-H), 7.46 (1H, s, Ar-H), 7.23 (2H, m, Ar-H), 6.99–7.09 (2H, m, Ar-H), 6.74 (2H, m, Ar-H), 4.00 (2H, m, $-CH_2-$), 3.93 (2H, m, $-CH_2-$), 2.12 (3H, s, $-CH_3$); LC–MS, *m*/*z*: 392.1 $(M+H)^+$. *Compound **7a***: Prepared according to the procedure described for the preparation of compound **6a**, starting from a mixture of 1-(2,4-difluorophenyl)-2-(1,2,4-triazol)-1-yl)propan-1-one (3.0 g, 0.01 mol), 10 g of a 50% aqueous soda lye and 1.5 mL tetrabutylammonium hydroxide in 15 mL toluene was stirred and heated to 50 °C. Then 1-bromo-3-(4-bromophenoxy)-propane (3.0 g, 0.01 mol) dissolved in 10 mL toluene, was instilled into the reaction solution. The mixture was subsequently stirred for 20 h at 50 °C (monitored by TLC, eluent, ethyl acetate/petroleum ether, 1/1, v/v). Post-treatment process of the reaction solution can according to the procedure described for the preparation of compound **6a**. The impure precipitated product was subsequently crystallized from the mixture solvent of ethyl acetate/petroleum ether (2:1, v/v) to afford compound **7a** (2.5 g) as white solid. Yield: 48.7%; mp: 120–122 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 9.57 (1H, s, TriazC_3-H), 8.35 (1H, s, TriazC_5-H), 6.92–7.37 (7H, m, Ar-H), 3.88 (2H, m, $-CH_2-$), 3.75 (2H, m, $-CH_2-$), 2.08 (3H, s, $-CH_3$), 2.01 (2H, m, $-CH_2-$); LC–MS, *m*/*z*: 450.1 $(M+H)^+$.
26. The crystal structure of 6c is well documented in our reported literature: Chen, J.; Wang, K.; Shen, Y.; Ren, L.; Chen, G. Acta Crystallogr., Sect. E 2011, E67, o2800. Full crystallographic details of 6c have been deposited at the Cambridge Crystallographic Data Center and allocated the deposition number CCDC 872815.
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