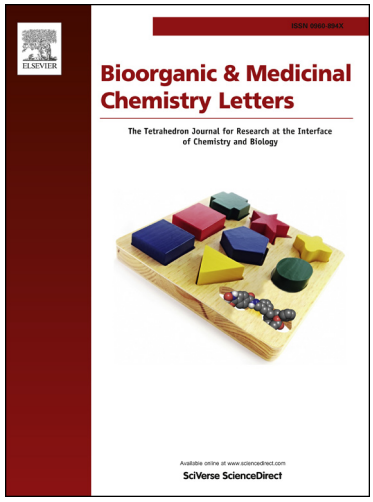

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Synthesis and evaluation of novel azoles as potent antifungal agents

Liangjing Li [a,b,†], Hao Ding [a,†], Baogang Wang[a], Shichong Yu[a], Yan Zou[a], Xiaoyun Chai[a,*], Qiuye Wu[a,*]

[a]*Department of Organic Chemistry, School of Pharmacy, Second Military Medical University, 325 Guohe Road, Shanghai 200433, China*

[b]*Cadet Company 2nd, Faculty of Naval Medicine, Second Military Medical University, 800 Xiangyin Road, Shanghai 200433, China*

Abstract: Using a rational approach to the design of antifungal agents, a series of azole agents with 1,3,4-oxadiazole side chains were designed and synthesised. The results of preliminary *in vitro* antifungal tests with eight human pathogenic compounds showed that all of the title compounds exhibited excellent activities against all of the tested fungi except *Aspergillus fumigatus*. Compounds **11e** and **11f** were found to be the most effective, with a minimum inhibitory concentration of 0.0039 μg/mL, followed by voriconazole, which has a MIC of 0.0625 μg/mL. The 1,3,4-oxadiazole side chain is not the major contributor but plays a role in eliciting the observed antifungal activity.

Fungal infections, which are an increasing healthcare concern worldwide, are associated with significant costs, morbidity, and mortality.[1-3] Although the number and types of antifungal drugs have markedly increased, only a few of the identified drugs can be used in the clinic.[4] The commonly used antifungal agents are azoles, polyenes, and echinocandins.[3,5] Among these antifungal drugs, the gold standard for

*Corresponding authors. Tel: +86 21 81871228 (Xiaoyun Chai), Tel./fax: +86 21 81871225 (Qiuye Wu).

E-mail addresses: chaixy1207@163.com (Xiaoyun Chai), wuqy640319@163.com (Qiuye Wu).

† These two authors contributed equally to this work.

the treatment of fungal infections is Amphotericin B (polyenes), which has been widely used in clinical practice and is effective against a large spectrum of fungi; however, its adverse side effects, such as infusional toxicity, nephrotoxicity, and low blood potassium levels, are serious.[6,7] Recently, resistance to echinocandin drugs for the treatment of fungal infections has increasingly emerged. Moreover, the expensive price of echinocandins also limits their application.[8,9] The above mentioned limitations emphasise the pressing need for novel antifungal agents. Azoles play a leading role in the treatment of invasive fungal infections. Based on their spectrum of activity, safety profiles, costs, potential toxicity, and some other aspects, azoles, which are the most commonly used antifungal drugs, present significant superiority and have broad development prospects. In addition, it is worthwhile for us to conduct further research.

Azoles target ergosterol biosynthesis through the inhibition of the fungal cytochrome P450 14α-demethylase (CYP51) via a mechanism in which the N-4 of the azole binds to the sixth coordination of the heme iron atom of the porphyrin in the substrate binding site of the enzyme. Thereby, ergosterol is depleted, and this depletion leads to the inhibition of either fungal cell death or growth.[3,10-12] Based on the structure of the active site of CYP51 and the results of structure-activity relationship studies, the 1,2,4-triazole ring and the 2,4-difluorophenyl group and hydroxyl groups are essential for this type of antifungal drugs. In addition, the side chain is also very important to achieve improved activities.[13]

Based on the abovementioned facts, we designed and synthesised a series of 1-(1*H*-1,2,4-triazol-1-yl)-2-(2,4-difluorophenyl)-3-{4-[3-((5-substituted phenyl-1,3,4 -oxadiazol-2-yl)thio)propyl]piperazin-1-yl}-propan-2-ols, which retained the classic skeleton of azole agents, and altered the side chains to find potent systemic antifungal agents with higher antifungal activity.

The synthetic route designed for the intermediates **7a-t** is outlined in **Scheme 1**. Compounds **7a-t** were synthesised from substituted benzoic acid through six steps.[14] Various substituted benzoic acids (**1a-t**) were treated with $SOCl_2$ to obtain compounds **2a-t**, which were reacted with CH_3OH and EtN_3 in CH_2Cl_2 at 0°C to yield

compounds **3a-t**. Compounds **4a-t** were prepared by the reaction of compounds **3a-t** with hydrazine hydrate in CH_3OH under reflux conditions for approximately 1 s. Subsequently, compounds **5a-t** were obtained by the reaction of compounds **4a-t** with CS_2 and KOH in CH_3OH. Compounds **6a-t** were obtained by the cyclization reaction of compounds **5a-t** in the presence of HCl at 0-5°C. Finally, treatment of compounds **6a-t** with 1,3-dibromopropane in the presence of K_2CO_3 in CH_3CN afforded compounds **7a-t** in moderate yields.

<Scheme 1.>

The chemical synthesis of the target compounds is outlined in **Scheme 2**. The coupling reaction between compound **8** and N-Boc-piperazine afforded compound **9**, which was stirred with CH_3COOF in CH_2Cl_2 to yield the key intermediate **10**. The target compounds **11a–t** were synthesised through the treatment of compound **10** and compounds **7a-t** in the presence of K_2CO_3, KI, and CH_3CN at 80°C. All of the novel compounds described above were characterised by ESI-MS and NMR spectroscopic analyses.

<Scheme 2.>

The *in vitro* minimal inhibitory concentrations (MICs) of the compounds were determined through the micro-broth dilution method in 96-well micro test plates according to the methods defined by the National Committee for Clinical Laboratory Standards (NCCLS).[15] The MIC_{80} was defined as the first well with an approximate 80% reduction in growth compared to the growth observed in the drug-free well. For the assays, the title compounds to be tested were dissolved in dimethyl sulfoxide (DMSO), serially diluted in growth medium, inoculated, and incubated at 35°C. The growth MIC was determined at 24 h for *Candida albicans* and at 72 h for *Cryptococcus neoformans*. The data presented are the means of three replicate tests performed with each antifungal agent. All of our susceptibility tests were performed three times for each antifungal agent.

<Table 1.>

The *in vitro* antifungal activities of the newly synthesised azole derivatives **11a**-**11t** and the reference drugs fluconazole (FCZ), ketoconazole (KCZ), itraconazole

(ICZ), and voriconazole (VCZ) against eight common clinical fungi are reported in **Table 1** . In general, the newly synthesised azole derivatives showed good antifungal activities except against *Aspergillus fumigatus*. The target compounds showed excellent activity against *Candida albicans.*, which has a worldwide distribution and is the most common cause of life-threatening fungal infections. Compounds **11c**, **11e, 11f**, and **11g** (with MIC_{80} values of 0.0039 μg/mL) are 64-fold more potent than ICZ (with an MIC_{80} value of 0.25 μg/mL) and 16-fold more potent than VCZ (with an MIC_{80} value of 0.0625 μg/mL). Compound **11i** showed the highest activity against *Candida glabrata* (with an MIC_{80} value of 0.0039 μg/mL) and was 16-, 32-, 256-, and 2051-fold more potent than VCZ, ICZ, FCZ, and KCZ, respectively. As a rule, the compounds with a halogen group (**11b-11j**) were found to be more potent than those compounds with alkyl, alkoxy, nitro, and cyano groups (**11k-11t**). We hypothesised that this decrease in activity could be ascribed to the electronic effects and the spatial configuration of the substituent.

This study was undertaken to evaluate the effects on the antifungal properties of novel azoles containing1,3,4-oxadiazole side chains. The data shown in Table 1 indicate that almost all of the target compounds have strong activities against eight common clinical fungi, with the exception of *Aspergillus fumigatus.* Compared with the intermediate **10,** the title compounds show markedly improved antifungal activity. This finding demonstrates that the 1,3,4-oxadiazole side chains are responsible for the antifungal activity of the title compounds and that the 1,3,4-oxadiazole side chain is an ideal supplemental group that presumably contributes to enzyme binding. In this context, the antifungal activity data presented in this manuscript suggest a potential role of the new compounds for the treatment of *Candida* infections and contribute to the active and challenging research on the development of novel antifungal agents. Compared with some first-line drugs, this series of novel antifungal compounds demonstrated improved antifungal activity in *in vitro* studies; thus, it is worthwhile for us to undertake further studies to identify the best compound. This study has laid a solid foundation for further lead compound optimisation through systematic chemical modifications of this class of compounds.

Acknowledgments

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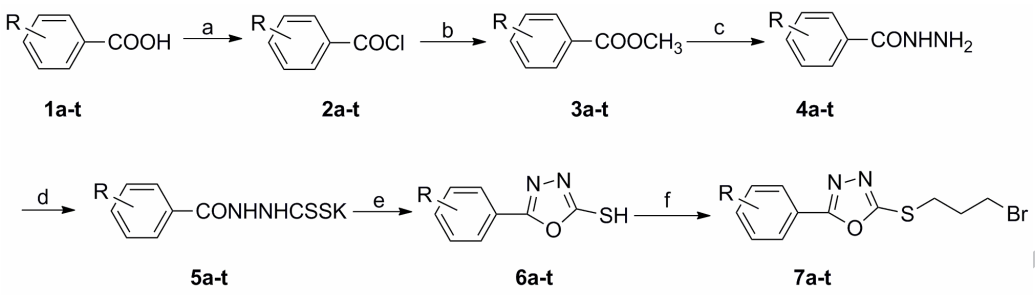
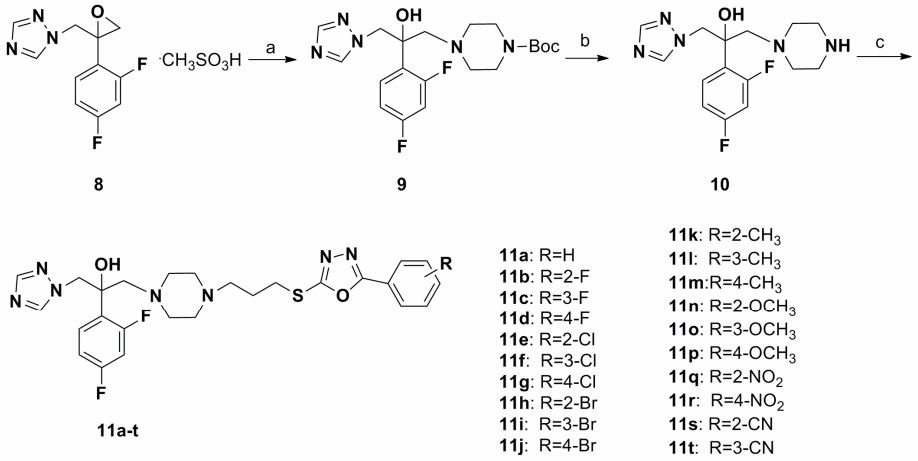


Scheme 1. Synthetic route to intermediates **7a-t**. Conditions and reagents: (a) $SOCl_2$, reflux, 5h; (b) CH_3OH, EtN_3, CH_2Cl_2, 0℃; (c) $NH_2NH_2{\cdot}H_2O$, CH_3OH, reflux, 5h; (d) CS_2, CH_3OH, KOH, rt-reflux; (e) HCl, 0-5℃; (f) $BrCH_2CH_2CH_2Br$, K_2CO_3, CH_3CN, rt, 8h.



Scheme 2. Synthetic route to the target compounds **11a-t**. Conditions and reagents: (a) N-Boc-piperazine, Et_3N, C_2H_5OH, reflux, 8h; (b) CF_3COOH, CH_2Cl_2, rt; (c) compounds **7a-t**, K_2CO_3, KI, CH_3CN, 80℃.

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

Compd.	R	*C.alb Y0109*	*C. alb SC5314*	*C. par*	*C. neo*	*C. gla*	*T. rub*	*A. fum*	*M. gyp*
11a	H	0.0156	0.0156	0.0625	0.0625	0.0625	0.25	>64	1
11b	2-F	0.0156	0.0625	0.25	0.0625	0.0625	0.0625	>64	1
11c	3-F	0.0625	0.0039	0.25	0.0625	0.0625	0.0625	>64	1
11d	4-F	0.25	0.0156	0.25	0.25	0.25	0.0625	>64	4
11e	2-Cl	0.0039	0.0039	0.25	0.25	0.25	0.25	>64	4
11f	3-Cl	0.0039	0.0039	0.25	0.0625	0.25	0.0625	>64	1
11g	4-Cl	0.0625	0.0039	0.0625	0.0625	0.0625	0.25	>64	4
11h	2-Br	0.0625	0.0156	0.0625	0.0625	0.0625	0.25	>64	0.25
11i	3-Br	0.0625	0.0625	0.25	0.25	0.0039	0.25	>64	1
11j	4-Br	0.0625	0.0625	0.0625	0.25	0.0156	0.25	>64	0.25
11k	2-CH_3	0.25	0.25	0.125	0.125	0.125	0.25	>64	0.25
11l	3-CH_3	0.25	0.125	4	1	1	1	>64	1
11m	4-CH_3	0.125	0.25	0.025	1	0.25	1	>64	1
11n	2-OCH_3	0.125	0.25	16	4	1	0.25	>64	16
11o	3-OCH_3	0.25	0.25	0.25	0.25	0.25	0.0625	>64	0.25
11p	4-OCH_3	0. 25	0.25	0.25	0.25	0.25	0.25	>64	0.25
11q	2-NO_2	0.25	0.125	0.125	0.125	0.25	0.25	>64	0.25
11r	4-NO_2	0.125	0.25	0.125	0.25	0.125	1	>64	1
11s	2-CN	0.125	0.125	0.25	0.25	0.125	0.25	>64	1
11t	3-CN	0.125	0.125	0. 25	0.25	0.25	0.25	>64	1
10	-	1	0.5	2	4	0.25	1	>64	16
KCZ	-	8	4	8	4	8	8	2	2
FCZ	-	0.5	0.5	1	1	1	>64	8	8
ICZ	-	0.25	0.25	0.125	0.125	0.125	0.5	1	1
VCZ	-	0.0625	0.0625	0.0625	0.0156	0.0625	0.25	0.0625	0.125

Abbreviations: *C. alb., Candida albicans; C. par., Candida parapsilosis; C. neo., Cryptococcus neoformans; C. gla., Candida glabrata; T. rub., Trichophyton rubrum; A.fum., Aspergillus fumigatus; M. gyp., Microsporum gypseum.*

Graphical abstract

Synthesis and evaluation of novel azoles as potent antifungal agents

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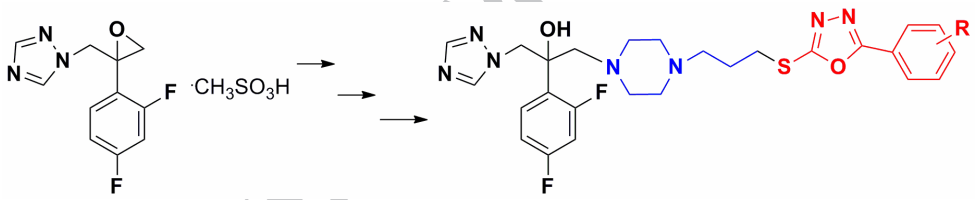
[a] *Department of Organic Chemistry, School of Pharmacy, Second Military Medical University, 325 Guohe Road, Shanghai 200433, China*

[b] *Cadet Company 2nd, Faculty of Naval Medicine, Second Military Medical University, 800 Xiangyin Road, Shanghai 200433, China*



Twenty novel azoles have been designed and synthesized as potential antifungal agents. All the title compounds exhibited excellent activity with a broad spectrum of antifungal activity.

*Corresponding authors. Tel: +86 21 81871228 (Xiaoyun Chai), Tel./fax: +86 21 81871225 (Qiuye Wu).

E-mail addresses: chaixy1207@163.com (Xiaoyun Chai), wuqy640319@163.com (Qiuye Wu).

† These two authors contributed equally to this work.