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Original article

Synthesis and biological evaluation of novel triazole derivatives as antifungal agents

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ABSTRACT

A series of 1-(benzylamino)-2-(2,4-difluorophenyl)-3-(1*H*-1,2,4-triazol-1-yl)propan-2-ols compounds were synthesized and evaluated for their antifungal activities *in vitro*. The results showed that compounds **6A** and **6B** exhibited good antifungal activity. Compound **6A8** showed the strongest antifungal activity, which was significantly higher than that of the lead compounds and positive-control drugs Fluconazole and Itraconazole. In particular, the antifungal activity of compound **6A8** against *Candida albicans* and *Candida krusei* (MIC80 both at 0.00097 μg/mL) was 515 and 64 times that of Fluconazole, respectively. The structure–activity relationships of the synthesized compounds were discussed, and the docking model of the target compounds with fungal lanosterol 14α-demethylase (CYP51) was analyzed.

1. Introduction

During the past two decades, the incidence and death rate of deep fungal infection have drastically increased, which has become a main cause of death in major diseases such as AIDS and tumors [1,2]. Such alarming data demonstrates the importance of antifungal drugs in clinical anti-infective treatment. Currently, azole antifungal drugs are widely used in clinical treatment [3]; in particular, Fluconazole, Itraconazole, Voriconazole, *etc*. (Fig. 1), are now preferred as first-line antifungal therapy [4,5]. Recently, new azole drugs such as Posaconazole and others have also entered the market [6]. However, the extensive use of azole antifungal drugs has led to the resistance of clinical pathogenic fungi to drug treatment, one of the main causes of clinical treatment failure [4,7]. Therefore, development of new azole drugs for clinical treatment is a pressing need.

Azole antifungal drugs block the biosynthesis of fungal cell membranes and suppress fungal growth by inhibiting CYP51 which is a critical enzyme in the ergosterol synthesis pathway [8]. Researches indicated that the triazole ring and 2,4-difluorophenyl group were the pharmacophores of antifungal agents. The 1,2,4-triazole ring binds to the Fe atom of the heme prosthetic group in the active cavity of the target enzyme, whereas 2,4-difluorophenyl binds to the hydrophobic pocket. The configuration of the remaining side chain varies considerably and it binds to the entry pathway of the target enzyme and can largely affect its ability to bind target enzyme [9].

Previous studies showed that 1-(benzyl(prop-2-ynyl)amino)-2-(2,4-difluorophenyl)-3-(1*H*-1,2,4-triazol-1-yl)propan-2-ol compounds (Fig. 2) exhibited good antifungal activity [10]. In this study, we designed and synthesized a series of derivatives of these compounds (Fig. 2) by replacing the aromatic ring on the side chain, changing the distance between the aromatic ring and the nitrogen atom and replacing different groups on the nitrogen. The antifungal activity of target compounds and their preliminary structure–activity relationships were investigated, and the docking model of the target compounds with CYP51 was analyzed.

2. Experimental

The synthesis of the target compounds was performed as shown in Scheme 1. Key intermediate **4** was synthesized *via* a known route [11]. Intermediate **4** was reacted with different alkylamines in the presence of triethylamine, then treated with concentrated HCl to provide the key intermediates **5** [10]. Lastly, in the presence of KI and K_2CO_3, intermediate **5** reacted with different substituted benzyl chlorides, and target compound **6** was obtained. Compounds **6A1**-**6A4** are known lead compounds and served as positive controls; the remaining 31 compounds were all new.

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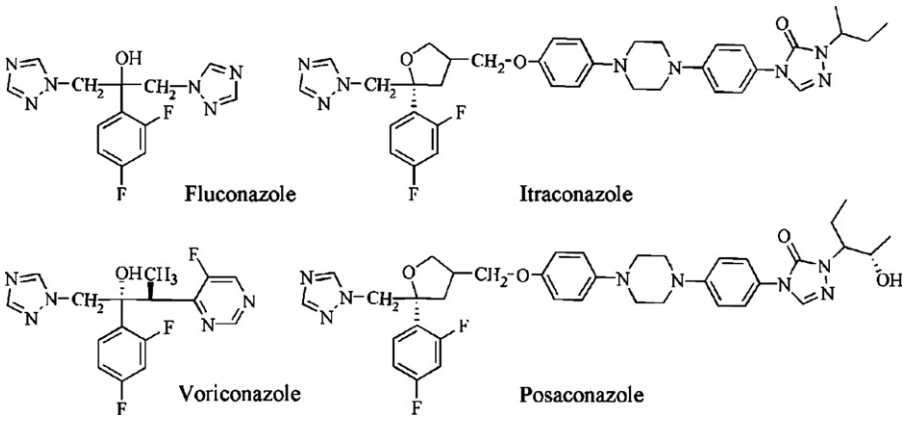


Fig. 1. Triazole antifungal drugs.

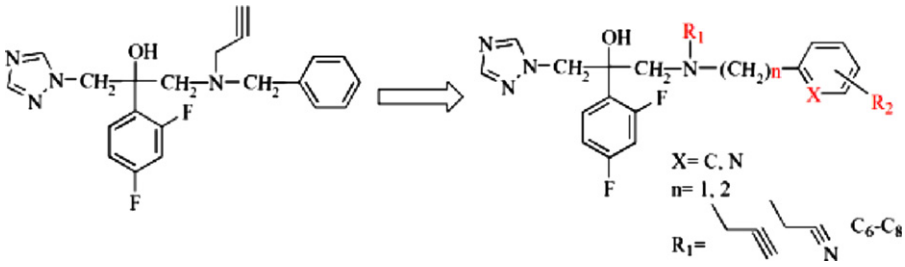


Fig. 2. The target compounds.

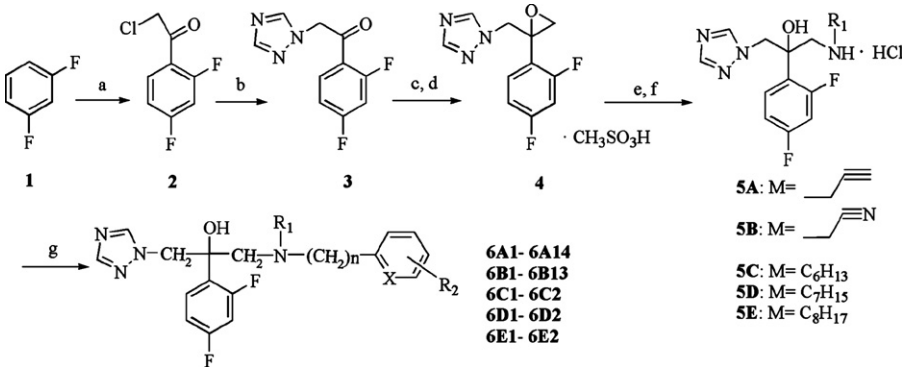
Supplementary data associates with this article can be found in supporting information.

3. Results and discussion

All the new target compounds were tested *in vitro* against seven common human pathogenic fungi [12,13], and the MIC_{80} values are shown in comparison with that of Fluconazole and Itraconazole in Table 1.

Compounds **6A1**–**6A4** were known lead compounds that showed good activity [10]. They were tested for antifungal activity for comparison purposes in our study. As shown in Table 1, the new compounds, **6A7**–**6A8**, **6A12**–**6A14**, **6B1**–**6B9**, **6B11**–**6B13** showed high-efficiency, broad-spectrum antifungal activity against six fungal strains (except *Aspergillus fumigatus*), compounds **6A5**–**6A6**, **6A9**–**6A11** and **6B10** showed middle-level antifungal activity and compounds **6C**, **6D**, **6E** showed low antifungal activity. Noticeably, the activity of compounds **6A7**–**6A8** was especially strong. Compound **6A8** exhibited significantly higher antifungal activity against most of the strains (including *A. fumigatus)*, than did the positive-control drugs Fluconazole and Itraconazole. In particular, the antifungal activity of compound **6A8** against *Candida albicans* and *C. krusei* was respectively 515 and 64 times that of Fluconazole and was several times stronger than that of lead compounds **6A1**–**6A4** as well.

As most **6A** compounds exhibited good *in vitro* antifungal activity, this supported our contention that replacing the side chain on the nitrogen with propargyl could indeed substantially increase the activity of the compound [10]. However, the antifungal activity of most **6B** compounds, although higher than Fluconazole, was noticeably lower than **6A** compounds. This indicated that replacing the side chain on nitrogen with cyanomethyl was worse for antifungal activity than replacing with propargyl. On the other hand, replacing the side chain on nitrogen with a long hydrocarbon chain almost eliminated the antifungal activity of the compound, which was demonstrated by



Scheme 1. The synthesis route of the target compounds. Reagents and conditions: (a) $ClCH_2COCl$, $AlCl_3$, 50 °C, 5 h, yield 85%; (b) 1*H*-1,2,4-triazole, K_2CO_3, $C_6H_5CH_3$, reflux, 5 h, yield 82%; (c) $(CH_3)_3SOI$, $NaHCO_3$, $C_6H_5CH_3$, 60 °C, 3 h; (d) CH_3SO_3H, 0 °C, 1 h, yield 75%; (e) alkylamine, Et_3N, CH_3CH_2OH, reflux, 5-6 h; (f) HCl (g), yield 80-90%; (g) substituted benzyl chloride, KI, K_2CO_3, CH_3CN, reflux, 5-6 h, yield 30-50%.

Table 1
The structure and *in vitro* antifungal activity (MIC_{80}, μg/mL) of target compounds.

No.	Compound structure				Antifungal activity (MIC_{80}, μg/mL)							
	R_1	n	X	R_2	*C. alb.* SC5314	*C. alb.* Y0109	*C. neo.*	*C. tro.*	*T. rub.*	*C. par.*	*C. kru.*	*A. fum.*
6A1		1	C	H	0.0039	0.0039	0.25	0.0625	1	0.0625	0.0039	64
6A2		1	C	4-F	0.0156	0.0156	0.0625	0.25	64	0.0625	0.0156	>64
6A3		1	C	3-F	0.0039	0.0156	0.0625	0.0625	64	0.0625	0.0156	64
6A4		1	C	2-F	0.0156	0.0625	0.0156	0.0625	16	0.0625	0.0156	>64
6A5		1	C	4-$C(CH_3)_3$	0.0156	4	0.25	1	16	16	0.0156	>64
6A6		1	C	4-CF_3	0.25	0.25	1	4	16	1	0.25	>64
6A7		1	C	3,4-2F	0.0039	0.0156	0.0625	0.25	16	0.0625	0.0156	64
6A8		1	C	2,4-2F	0.00097	0.00097	0.0156	0.25	1	0.0156	0.0039	16
6A9		1	C	3,4-2Cl	0.25	0.25	1	4	16	1	0.25	>64
6A10		1	C	2,3-2Cl	0.25	0.25	0.0625	16	16	0.25	1	>64
6A11		1	N	H	0.25	0.25	0.0625	1	64	0.25	0.0156	>64
6A12		2	C	4-F	0.0625	0.0625	0.0625	4	64	1	0.0625	>64
6A13		2	C	3-F	0.0625	0.0625	0.0625	1	>64	0.25	0.0625	>64
6A14		2	C	2-F	0.0625	0.0625	0.0156	0.25	16	0.25	0.0156	64
6B1		1	C	H	0.25	0.25	0.0625	1	64	0.25	0.25	>64
6B2		1	C	4-F	0.0625	0.0625	0.0625	0.25	>64	0.25	0.0625	>64
6B3		1	C	3-F	0.0156	0.0625	0.0625	0.25	16	0.0625	0.0156	>64
6B4		1	C	2-F	0.0625	0.0625	0.0156	1	64	0.25	0.0625	>64
6B5		1	C	4-Cl	0.0156	0.0156	0.0625	0.25	16	0.0625	0.0156	64
6B6		1	C	4-OCH_3	0.0625	0.0625	0.0625	4	16	0.25	0.0625	>64
6B7		1	C	4-CH_3	0.0625	0.0625	0.25	1	>64	0.25	0.0625	>64
6B8		1	C	3,4-2F	0.0625	0.0625	0.25	0.25	>64	0.25	0.0625	>64
6B9		1	C	2,4-2F	0.0625	0.0625	0.0625	0.25	4	0.0625	0.0625	64
6B10		1	N	H	0.25	0.25	0.25	4	64	4	0.0625	>64
6B11		2	C	4-F	0.0156	0.0156	0.0625	0.25	0.0156	0.0625	0.0156	64
6B12		2	C	3-F	0.0625	0.0625	0.0625	4	0.0625	0.25	0.0625	>64
6B13		2	C	2-F	0.0625	0.0156	0.0625	0.25	64	0.25	0.0625	>64
6C1	C_6H_{13}	1	C	4-F	16	4	/	64	64	16	1	>64
6C2	C_6H_{13}	1	C	4-Cl	4	4	/	4	64	16	1	>64
6D1	C_7H_{15}	1	C	4-F	4	4	/	4	64	16	1	>64
6D2	C_7H_{15}	1	C	4-Cl	1	1	/	16	64	4	1	>64
6E1	C_8H_{17}	1	C	4-F	4	4	/	64	64	64	1	>64
6E2	C_8H_{17}	1	C	4-Cl	1	1	/	4	64	4	1	>64
Fluconazole	/	/	/	/	0.5	0.5	0.25	1	0.25	0.25	0.25	>64
Itraconazole	/	/	/	/	0.0625	0.0625	0.0625	0.0625	0.25	0.0156	0.0625	4

C. alb., SC5314 and Y0109, *Candida albicans*; *C. neo.*, *Cryptococcus neoformans*; *C. tro.*, *Candida tropicalis*; *T. rub.*, *Trichophyton rubrum*; *C. par.*, *Candida parapsilosis*; *C. kru.*, *Candida krusei*; *A. fum.*, *Aspergillus fumigatus*.

the low antifungal activity of compounds **6C**, **6D** and **6E**. Combining the results from this study and those from previous research [10,14,15], we proposed that the optimal strategy to maximize the antifungal activity of compound was to replace the side chain on nitrogen with a group of three carbon atoms.

The substituted group on the side chain benzene ring also contributed significantly to the compound activity. Compounds **6A7**–**6A8** showed highest activity, indicating that the difluoro-substituted group was especially favorable for antifungal activity. In addition, based on the activity comparisons between **6A11** and **6A1**, **6B10** and **6B1**, we obtained a preliminary conclusion that to replace the side-chain benzene ring with a heteroaromatic ring was unfavorable for antifungal activity. Based on the activity comparisons between **6A12**–**6A14** and **6A2**–**6A4**, and **6B11**–**6B13** and **6B2**–**6B4**, we found that to increase the distance between the side-chain benzene ring and

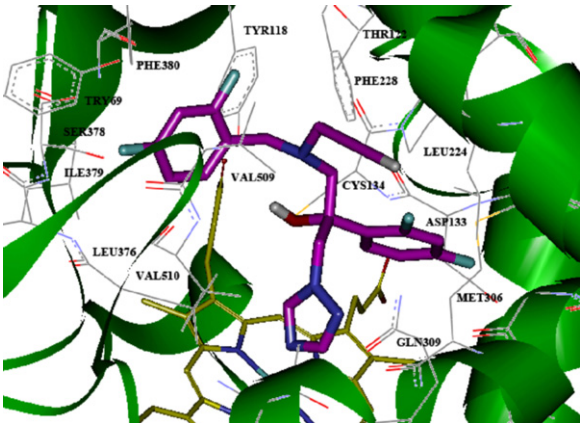


Fig. 3. The docking model for compound **6A8** and CYP51.

the nitrogen atom was slightly unfavorable for antifungal activity.

The binding modes of **6A8** in the active site of CYP51 of *C. albicans* showed (Fig. 3) that the triazole interacts with iron of the heme group, while the difluorophenyl group in the designed compound could be placed into the hydrophobic pocket formed by Met306, ASP133 and CYS134 and the 2,4-difluorobenzyl group would generate Π–Π stacking interactions with the Tyr118. The *N*-substituted group of the linker would be oriented to interact with a hydrophobic pocket formed by VAL510, LEU376, SER378, PHE380, VAL509 and TYR69.

4. Conclusion

In response to an urgent need for the discovery of novel antifungal agents, a series of 1-(benzylamino)-2-(2,4-difluorophenyl)-3-(1*H*-1,2,4-triazol-1-yl)propan-2-ols were designed and synthesized. The *in vitro* antifungal activity assay indicates that with propargyl or cyanomethyl as nitrogen side chain (compounds **6A** and **6B**) exhibited good antifungal activity. In particular, compound **6A8** showed optimal biologic activity against *C. albicans* (MIC_{80} = 0.00097 μg/mL) which was significantly stronger than that of the lead compounds and positive-control drugs. The side chain on nitrogen with propargyl of compounds is important for their antifungal activity. Replacing the side chain on nitrogen with cyanomethyl was worse for antifungal activity than replacing with propargyl and replacing the side chain on nitrogen with a long hydrocarbon chain almost eliminated the antifungal activity of the compound. Compound **6A8** provides a good starting point for further structural optimization.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.cclet.2013.01.015.

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