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Original article

Design and synthesis of novel triazole derivatives containing γ-lactam as potential antifungal agents

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ABSTRACT

A series of novel triazole derivatives containing γ-lactam were designed and synthesized, and their structures were confirmed by ^{1}H NMR, ^{13}C NMR and HRMS. The *in vitro* antifungal activities of the target compounds were evaluated. The results showed that all of the compounds exhibited stronger activity against the six clinically important fungi tested than fluconazole. **3D** and **3E** showed comparative activity against the fungi tested except for *Candida glabrata* and *Aspergillus fumigatus* as voriconazole. In addition, the docking model for **2A** and CYP51 was investigated.

1. Introduction

In recent years, the population of immunocompromised individuals, such as AIDS and organ transplants recipient, is increasing. This fact makes the invasive fungal infection become a global threat to human health [1,2]. Among the drugs to treat fungal infection, triazole derivatives as potent and safe antifungal agents have attracted attention for a long time [3,4]. Fluconazole, voriconazole, and itraconazole (Fig. 1) have been widely used in clinical therapy. But some impediments of these drugs still remain to be resolved. Firstly, with the extensive use of triazole antifungal drugs, the rate of drug resistance mutation is also increasing gradually [5,6]. Secondly, the poor water-solubility and drug–drug interactions are a common problem of triazole antifungal drugs [7]. Therefore, the discovery and development of novel antifungal agents for clinical treatment has important realistic meanings.

Albaconazole (Fig. 1) is one of the most potent antifungal agents reported, which has potent activity against many fungi such as *Candida* spp and *Aspergillus* spp [8]. A quinazolinone unit rather than a pyrimidine unit of voriconazole is the structure characteristic of albaconazole. Literatures have disclosed the important interaction between the carbonyl of quinazolinone unit with the His310 of CYP51, the target enzyme of triazole antifungal agents [9]. Our lab also has done some work in this area [10,11]. For example, the pyridine-substituted analogues of itraconazole, some of which showed good activities against pathogenic fungi. Unfortunately, all of those derivatives have poor water-solubility. Our previous studies found that the triazole derivatives containing 4,5,6,7-tetrahydrothiazolo[5,4-c]pyridine motif have good activities against *Candida* spp *in vitro* [12]. Compound **1** (Fig. 2) has the most potent activity among those derivatives. What is important is that the disulfate salt of compound **1** has good water-solubility, which can be given through intravenous injection.

Herein, we designed and synthesized two series of triazole derivatives featuring 5,6-dihydro-4*H*-pyrrolo[3,4-d]thiazol-4-one moiety **2** and 4,5-dihydro-6*H*-pyrrolo[3,4-d]thiazol-6-one moiety **3** (Fig. 2) on the basis of the structure characteristics of **1** and albaconazole. We hypothesized that the carbonyl of γ-lactam could interact with the His310 of CYP51, which can enhance the antifungal activity.

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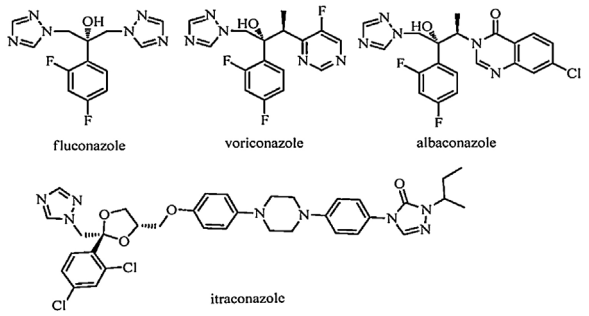


Fig. 1. Chemical structure of fluconazole, voriconazole, albaconazole, and itraconazole.

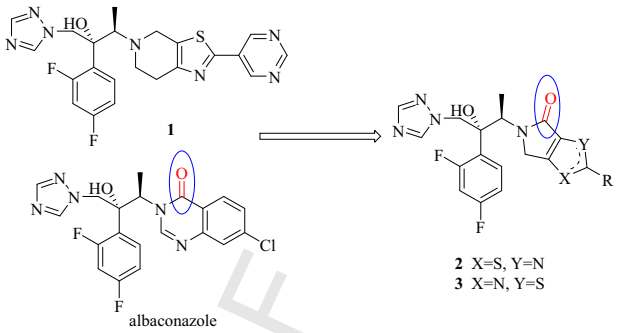


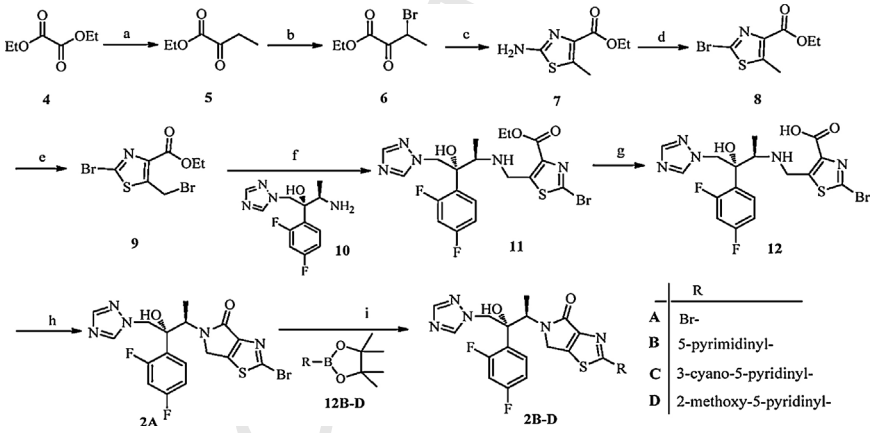
Fig. 2. The design of title compounds.

2. Experimental

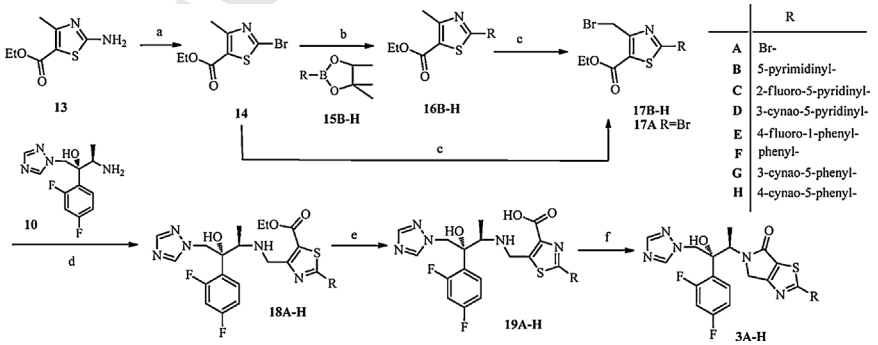
The synthetic route of title compounds **2A–D** is outlined in Scheme 1. The reaction of compound **4** with ethylmagnesium bromide yielded **5**. Then compound **5** reacted with $CuBr_2$ to give **6**, which underwent annelation to obtain **7**. Compound **7** underwent a Sandemyer reaction and bromination by NBS sequentially to afford **9**. The key intermediate **9** reacted with compound **10** [13] to give **11**, which underwent a hydrolysis and condensation to obtain compound **2A**. Compounds **2B–D** were obtained by treating **2A** with different boronic acid pinacol ester **12B–D** in 1, 4-dioxane and water.

The synthetic route of compounds **3A–H** is showed in Scheme 2. Commercially available compound ethyl 2-amino-4-methylthiazole-5-carboxylate (**13**) underwent a Sandemyer reaction to give compound **14**, which was treated with different boronic acid pinacol ester **15B–H** in 1, 4-dioxane and water, giving compounds **16B–H**. Compounds **16B–H** and **14** reacted with NBS in the solution of CCl_4 to yield compounds **17A–H**. With the important intermediates **17A–H** in hand, the title compounds **3A–H** can be formed easily through the similar procedures outlined in Scheme 1.

In vitro minimal inhibitory concentrations (MIC) of the title compounds were determined using the serial dilution method in 96-well microtest plates. The six pathogenic fungi were obtained from ATCC or clinic isolates. The MIC determination was performed according to Clinical and Laboratory Standards Institute (CLSI) guidelines [14,15]. All of test compounds were dissolved in DMSO serially diluted in growth medium.



Scheme 1. Synthetic route for title compounds **2A–D.** Reagents and conditions: (a) ethylmagnesium bromide, THF, −78 °C then −10 °C, 2 h; (b) $CuBr_2$, EtOAc/$CHCl_3$, reflux, 12 h; (c) thiourea, EtOH, reflux 2 h then r.t., 12 h; (d) ^tBuONO, $CuBr_2$, CH_3CN, r.t. then 60 °C, 6 h; (e) NBS, BPO, CCl_4, 60 °C, 12 h; (f) **9**, K_2CO_3, CH_3CN, 0 °C then r.t. overnight; (g) NaOH, THF, MeOH, 0 °C then r.t. overnight; (h) EDCI, HOBt, TEA, CH_2Cl_2, 0 °C then r.t. 2 h; (i) **12B–D**, Pd[$P(Ph_3)_4$], Cs_2CO_3, 1, 4-dioxane/H_2O,70 °C, 12 h.



Scheme 2. Synthetic route for title compounds **3A–H**. Reagents and conditions: (a) ^tBuONO, $CuBr_2$, DMF, CH_3CN, 0 °C then 60 °C, 1 h; (b) **15B–H**, Cs_2CO_3, $Pd(PPh_3)_4$, 1, 4-dioxane/H_2O, 80 °C, 12 h; (c) NBS, AIBN, CCl_4, r.t., overnight; (d) K_2CO_3, CH_3CN, 0 °C then r.t. overnight; (e) NaOH, MeOH, THF, 0 °C then r.t. 8 h; (f) EDCI, HOBt, TEA, 0 °C then r.t. 2 h.

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3. Results and discussion

As outlined in the Schemes 1 and 2, we can obtain the title compounds **2A–D** and **3A–H** smoothly and efficiently. Their structures were confirmed by ^{1}H NMR, ^{13}C NMR and HRMS (Supplementary data). The MIC_{80} values of the title compounds are showed in Table 1. Our previous experiments showed that DMSO has no influence on the growth of the fungi tested under the test conditions. So, the results of blank control experiments (DMSO only) are not showed in this table.

All the target compounds showed stronger activity against *Candida* spp than fluconazole. And especially some compounds (**3D**, **3E**, and **3F**) have comparative MIC_{80} values against all the tested fungi as voriconazole except for *Candida glabrata* and *Aspergillus fumigatus*. What is important is that some compounds of series **3** (**3A**, **3C**, **3D**, **3E**, **3F**) showed good activities against *Cryptococcus neoformans* which has been increasing prevalence during the last few decades [16]. In the overall view, the compounds of series **3** showed more potent activity than compounds of series **2**. Furthermore, the simple bromo-substituted compounds (**2A** and **3A**) demonstrated stronger activity against all the six fungi than other aromatic substituted compounds (**2B–D** and **3B–H)** except for **3E**. In addition, the different aromatic rings (**2B** and **2C**, **3D** and **3F**) have little influence on the activity, which can be explained by the fact that the conformation of the ligand tunnels is compatible with different ligands [17].

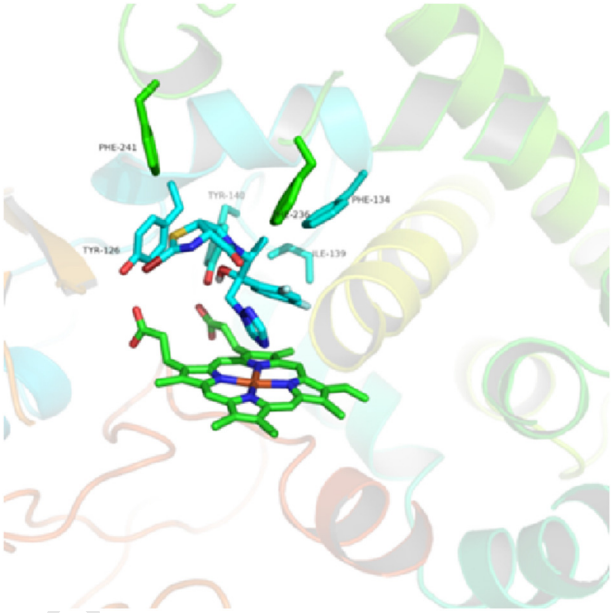
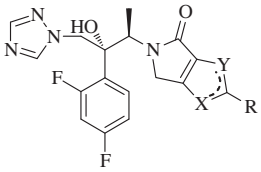
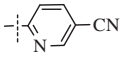
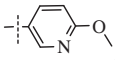
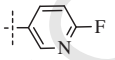
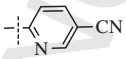
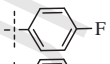
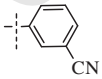
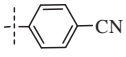


Fig. 3. The docking model for compound **2A** and CYP51.

Table 1
In vitro antifungal activity of the title compounds (MIC_{80}, μg/mL).



Compound	X	Y	R	*C.alb* Y0109	*C.alb* SC5314	*C.par*	*C.gla*	*C.neo*	*A.fum*
2A	S	N	-Br	0.0625	0.125	0.125	1	0.125	16
2B	S	N		0.125	0.03125	0.125	0.5	0.125	32
2C	S	N		0.125	0.03125	0.25	1	0.25	32
2D	S	N		0.25	0.125	0.125	0.5	0.125	64
3A	N	S	-Br	0.03125	0.03125	0.0625	0.25	0.0156	4
3B	N	S		0.125	0.125	0.125	1	1	16
3C	N	S		0.0625	0.03125	0.03125	0.25	0.03125	16
3D	N	S		0.03125	0.0625	0.03125	0.125	0.03125	32
3E	N	S		0.0156	0.0156	0.03125	0.125	0.03125	4
3F	N	S		0.03125	0.03125	0.03125	0.5	0.03125	4
3G	N	S		0.0625	0.0625	0.125	0.5	1	8
3H	N	S		0.0625	0.03125	0.125	0.25	0.125	8
FLZ	–	–	–	0.25	0.25	1	1	0.25	>64
VCZ	–	–	–	0.0156	0.0156	0.0156	0.0156	0.0156	0.25

Abbreviation: C.alb Y0109, Candida albicans Y0109; C.alb SC5314, Candida albicans SC5314; C.par, Candida parapsilosis; C.gla, Candida glabrata; C.neo, Cryptococcus neoformans; A.fum, Aspergillus fumigatus; FLU, Fluconazole; VCZ, Voriconazole.

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The docking model of representative compound **2A** and fungal CYP51 [18] was investigated using the GOLD Suite v5.0 software package (Fig. 3). Like the other triazole compounds, the 4-N of the triazole unit has a van Der Waals interaction with the heme iron. In addition, we can see that the 5, 6-dihydro-4*H*-pyrrolo[3,4-d]thiazol-4-one unit was located in the hydrophobic pocket composed of Phe-241, Phe-236, Ile-139 and Try-140. Furthermore, the bromine atom of **2A** have a halogen bond with Ser-382 (not showed), which can explain the potent activities of **2A** and **3A**.

4. Conclusion

In summary, a series of novel triazole derivatives containing γ-lactam were designed and synthesized. Their *in vitro* antifungal activities against six pathogenic fungi were evaluated. According to the results, the R group of the thiazole ring was well tolerated *in vitro*. For example, both the pyridyl-substituted compound **3D** and phenyl-substituted compound **3E** exhibited good activity against the *Candida* spp and *Cryptococcus neoformans* tested. However, the activities of these novel triazole derivatives against *Aspergillus fumigatus* are moderate and the further studies to improve activity against *Aspergillus fumigatus* are ongoing in our lab. In addition, the cytotoxicities and metabolic properties of these novel triazole derivatives will be determined in due time.

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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.2016.01.040.

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