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Design, synthesis, and structure-activity relationship studies of novel triazole agents with strong antifungal activity against *Aspergillus fumigatus*

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ABSTRACT

The incidence of invasive fungal infections has dramatically increased for several decades. In order to discover novel antifungal agents with broad spectrum and anti-*Aspergillus* efficacy, a series of novel triazole derivatives containing 1,2,3-benzotriazin-4-one was designed and synthesized. Most of the compounds exhibited stronger *in vitro* antifungal activities against tested fungi than fluconazole. Moreover, **6m** showed comparable antifungal activity against seven pathogenic strains as voriconazole and albaconazole, especially against *Aspergillus fumigatus* (MIC = 0.25 μg/ml), and displayed moderate antifungal activity against fluconazole-resistant strains of *Candida albicans*. A clear SAR study indicated that compounds with groups at the 7-position resulted in novel antifungal triazoles with more effectiveness and a broader-spectrum.

Over the past decades, invasive fungal infections (IFIs) have been dramatically increasing and presenting a severe threat to human health.[1–4] Clinically, the three major pathogens, including the *Candida albicans*, *Cryptococcus neoformans* and *Aspergillus fumigatus*, account for most of IFIs, which continue to be a major cause of morbidity and mortality in immunocompromised or HIV-infected patients.[5] The therapeutic antifungal agents available for the treatment of IFIs can be divided into four classes: polyenes (e.g. amphotericin B), echinocandins (e.g. caspofungin and micafungin), nucleoside analogues (e.g. 5-fluorocytosine) and azoles (e.g. fluconazole and itraconazole).

Among these antifungal agents, azoles exert activity by selective inhibition the lanosterol 14α-demethylase (CYP51), the key enzyme in sterol biosynthesis of fungi.[6] Most azoles including fluconazole, itraconazole, voriconazole, and posaconazole (Fig.1) are orally active, and show a broad-spectrum against most yeasts and filamentous fungi.[7] Due to their broader antifungal spectrum, higher efficacy and lower toxicity, azole drugs have been used as first-line antifungal agents.

Unfortunately, the increasing number of invasive *aspergillosis* among immunocompromised patients has increased dramatically.[8] Pulmonary infections with *Aspergillus fumigatus* can cause progressive dysphagia in severely ill patients.[9] However, this pathogen is intrinsically resistant to fluconazole, which is the most widely used azole in clinic. Therefore, it is urgent to develop novel antifungal azoles with excellent activity against pathogenic fungi, particularly to further enhance the anti-*Aspergillus* efficacy.

Albaconazole is a new oral triazole antifungal agent with good pharmacokinetics and excellent oral bioavailability.[10,11] It has demonstrated high *in vitro* activity against pathogenic yeasts and filamentous fungi, especially *Aspergillus fumigatus*, and has been shown to be effective in animal models.[11] Albaconazole has been studies in several clinical trials, including phase I and II studies on candidal vulvovaginitis, tinea pedis, and onychomycosis and no side effects have been published.[12,13] However, fewer literatures of structural modification to albaconazole have been reported than that to fluconazole.

As part of our research program focused on synthesis novel broad-spectrum and anti-*Aspergillus* antifungal agents, we aimed to develop a series of structural analogue of albaconazole. A quinazolinone unit is the structure characteristic of albaconazole and is the bioisostere of 1,2,3-benzotriazin-4-one. 1,2,3-benzotriazin-4-one is an important component of nitrogen-containing heterocycle and has attracted much attention in both the medicinal and agrochemical fields.[14–16] For example, many pharmacological compounds have been reported, including agents with antitumor, antiarthritic, diuretic and antitubercular activities.[17–20] Inspired by the bioisosteric replacement of quinazolinone moiety with 1,2,3-benzotriazin-4-one, a series of novel albaconazole analogues was designed and synthesized.

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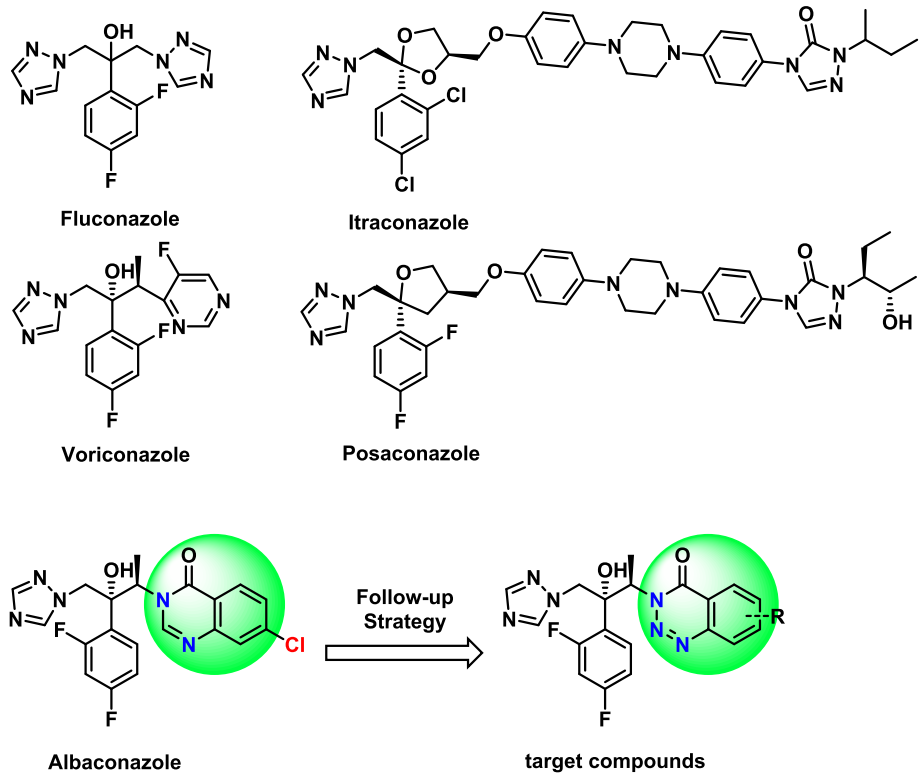

Fig. 1. Structures of clinic triazole antifungal agents and the designed compounds.

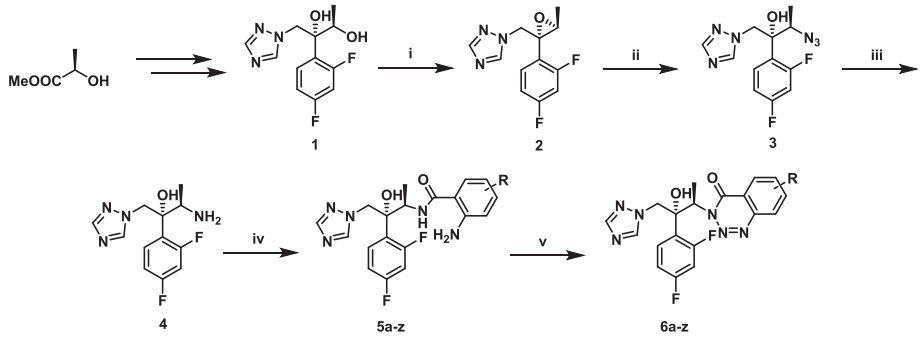

Fig. 2. Synthesis of the target compounds. (i) a) MsCl, Et_3N, DCM, 0 °C, 30 min; b) NaOH, H_2O, 0 °C, 2 h; (ii) NaN_3, NH_4Cl, DMF, 100 °C, 8 h; (iii) H_2, Pd/C, MeOH, rt, 4 h; (iv) Substututed anthranilic acids, DIEA, PyBOP, DMF, rt, 2 h; (v) Tertbutyl nitrite, MeCN, 0 °C, 2 h.

All compounds in this study were prepared following the general strategies illustrated in Fig. 2. Key intermediate **1** was prepared following the procedure established in the literature.[21] Then, triazolyldiol **1** was formed methanesulfonate and then eliminated to yield epoxide **2**. The ring-opening reaction of epoxide **2** with NaN_3 followed by catalytic hydrogenation to give amino **4**, which was then subjected to the amidation reaction conditions with commercially available substituted anthranilic acid. Finally, the diazotization on the amino group was coupled to the amide to form the target compounds **6a-z**.

The *in vitro* antifungal activities of target compounds **6a-z** were evaluated according to the protocols from the NCCLS.[22,23] The broth microdilution method was used to determine the minimum inhibitory concentration (MIC) of the target compounds in 96-well microtest plates. Fluconazole, voriconazole and albaconazole were used as reference drugs. Previous experiments showed that DMSO has no influence on the growth of the fungi tested under the test conditions.

As shown in Table 1, most of the target compounds showed strong activities against *Candida albicans* and *Cryptococcus neoformans* with MIC values in the range of 0.0156 to 2.0 μg/ml. What is important is that compounds **6l-s**, **6w** and **6z** exhibited good activities against *Aspergillus fumigatus*. Subsequently, compounds **6l-s**, **6w** and **6z** were selected out in order to further evaluate the antifungal spectrum as showed in Table 2. According to the results, these compounds possessed excellent antifungal activities against all strains tested. Among these compounds, 7-Cl substituted analogue **6m** displayed the most remarkable *in vitro* activity against all of the tested strains, especially with the MIC value of 0.25 μg/ml against *Aspergillus fumigatus* and *Microsporum gypseum* which was superior to fluconazole and was comparable to voriconazole and albaconazole.

Due to the widespread treatment use of fluconazole in the clinic, fluconazole-resistance for treating fungal infections was seriously increasing. Based on the results of *in vitro* antifungal activities assays. Compound **6l-o** were further evaluated against fluconazole-resistant strains of *Candida albicans* 100 and 103. As shown in Table 3,

Table 1
In vitro antifungal activity of the target compounds against three pathogenic fungi (MIC, μg/ml).

Compd.	R	*C.alb.* SC5314	*C.neo.* 32,605	*A.fum.* 7544
6a	5-F	0.5	0.0625	> 64.0
6b	5-Cl	0.25	0.0625	32.0
6c	5-Br	0.25	0.0625	32.0
6d	6-F	0.0625	0.25	16.0
6e	6-Cl	0.0313	0.5	> 64.0
6f	6-Br	0.125	2.0	> 64.0
6g	6-I	0.125	0.5	32.0
6h	6-OCH_3	0.0625	1.0	32.0
6i	6-CF_3	0.25	2.0	> 64.0
6j	6-NO_2	0.5	2.0	> 64.0
6k	6-CH_3	0.0625	0.25	16.0
6l	7-F	0.0156	0.25	1.0
6m	7-Cl	0.0156	0.0313	0.25
6n	7-Br	0.0156	0.0625	0.5
6o	7-I	0.0156	0.125	1.0
6p	7-OCH_3	0.0156	0.125	2.0
6q	7-CF_3	0.0156	0.125	4.0
6r	7-NO_2	0.0156	0.25	4.0
6s	7-CH_3	0.0156	0.125	2.0
6t	8-F	0.5	0.0313	> 64.0
6u	8-Cl	0.25	0.25	> 64.0
6v	8-Br	0.5	0.25	> 64.0
6w	6,7-2F	0.0313	0.5	2.0
6x	6-F-7-Cl	0.125	0.25	8.0
6y	Naphthalene	0.125	0.5	> 64.0
6z	H	0.0313	0.25	4.0
FCZ	/	0.5	0.5	> 64.0
VCZ	/	0.0313	0.0625	0.25
ALB	/	0.0313	0.0313	0.25

Abbreviations: C.alb.: *Candida albicans; C.neo.*: *Cryptococcus neoformans; A.fum.*: *Aspergillus fumigatus;* FCZ: fluconazole; VCZ: voriconazole; ALB: albaconazole.

Table 2
In vitro antifungal activity of the selected target compounds against other four pathogenic strains (MIC, μg/ml).

Compd.	R	*C. alb.* Y0109	*C. par.* 22,019	*C. gla.* 537	*M. gyp.* cmcc
6l	7-F	0.0156	0.0313	0.0313	2.0
6m	7-Cl	0.0156	0.0156	0.0156	0.25
6n	7-Br	0.0156	0.0313	0.0156	0.5
6o	7-I	0.0156	0.0625	0.0313	1.0
6p	7-OCH3	0.0156	0.0313	0.0156	2.0
6q	7-CF3	0.0156	0.0313	0.0313	2.0
6r	7-NO2	0.0156	0.0313	0.0313	4.0
6s	7-CH3	0.0156	0.0313	0.0156	2.0
6w	6,7-2F	0.0156	0.0313	0.0625	1.0
6z	H	0.0156	0.0313	0.0625	2.0
FCZ	/	0.5	1.0	2.0	32.0
VCZ	/	0.0156	0.0156	0.0156	0.25
ALB	/	0.0156	0.0156	0.0625	1.0

Abbreviations: C. alb.: *Candida albicans*; *C. par.*: *Candida parapsilosis*; *C. gla.*: *Candida glabrata*; *M. gyp.*: *Microsporum gypseum*; FCZ: fluconazole; VCZ: voriconazole; ALB: albaconazole.

compound **6m** showed moderate antifungal activity with MIC values in the range of 2.0 to 8.0 μg/ml. This result was comparable to the albaconazole, and was superior to fluconazole and voriconazole.

The structure-activity relationship between various substituents on the 1,2,3-benzotriazin-4-one was investigated. In the overall view, compounds with groups at the 7-position exhibited more potent and broader-spectrum antifungal activities than that with groups at the 5-, 6- and 8-position. Furthermore, compounds with electron-withdrawing groups at the 7-position such as –NO_2 (**6r**) and –CF_3 (**6q**) resulted in a decrease against *Aspergillus fumigatus* and *Microsporum gypseum*. In addition, compounds **6l-o** and **6w** with halogens showed higher antifungal activities than those with electron-withdrawing groups. Moreover, Compound **6y** which replaced 1,2,3-benzotriazin-4-one with 1,2,3-naphthotriazin-4-one showed no advantage than **6z**. Finally, 7-Cl substituted analogue **6m** displayed the most remarkable *in vitro* activity against all of the tested strains, especially with the MIC value of 0.25 μg/ml against *Aspergillus fumigatus* and *Microsporum gypseum*.

Table 3
In vitro antifungal activity of the target compounds against fluconazole-resistant strains (MIC, μg/ml).

Compd.	R	*C. alb.* 100	*C. alb.* 103
6l	7-F	32.0	64.0
6m	7-Cl	8.0	2.0
6n	7-Br	16.0	32.0
6o	7-I	> 64.0	> 64.0
FCZ	/	> 64.0	> 64.0
VCZ	/	> 64.0	> 64.0
ALB	/	8.0	4.0

Abbreviations: C. alb. 100: The fluconazole-resistant *Candida albicans* isolate 100; *C. alb.* 103: The fluconazole-resistant *Candida albicans* isolate 103; FCZ: fluconazole; VCZ: voriconazole; ALB: albaconazole.

In order to understand the binding mode of compound **6m**, docking simulations were carried out using the Surflex-Dock program in the SYBYL-X 2.0 software. The published crystal structures of *C. albicans* CYP51 (PDB ID: 5TZ1) and *A. fumigatus* CYP51 (PDB ID: 4UYM) served as a useful template for generating binding modes.[24,25] Images depicting the proposed binding modes were generated using PyMOL. As showed in Fig. 3A, the triazole ring and difluorophenyl group of **6m** formed a coordination bond and hydrophobic interactions with heme group and Phe-126 and Tyr-132, respectively. The 1,2,3-benzotriazin-4-one side chain extended into the CYP51 channel to form favorable hydrophobic interactions and *van der Waals* with the surrounding residues such as Tyr-118, Leu-121, Phe-233, Phe-380 and Met-508. The binding mode of **6m** with *A. fumigatus* CYP51 was similar to that with *C. albicans* CYP51 (Fig. 3B).

The incidence of invasive fungal infections has dramatically increased for several decades, especially the increasing number of invasive *aspergillosis* among immunocompromised patients. However, this pathogen is intrinsically resistant to fluconazole. A major focus of our optimization effort was to broaden antifungal spectrum and increase the anti-*Aspergillus* efficacy. In this report we have designed and synthesized a series of novel triazole derivatives containing 1,2,3-benzotriazin-4-one and their *in vitro* antifungal activities were evaluated. Most of the compounds exhibited stronger antifungal activities against *Candida albicans* and *Cryptococcus neoformans* with MIC values in the range of 0.0156–2.0 μg/ml than fluconazole. Moreover, **6m** showed comparable antifungal activity against seven pathogenic strains as voriconazole and albaconazole, especially against *Aspergillus fumigatus* (MIC = 0.25 μg/ml), and displayed moderate antifungal activity against fluconazole-resistant strains of *Candida albicans*. A clear SAR study indicated that compounds with groups at the 7-position resulted in novel antifungal triazoles with more effectiveness and a broader-spectrum. The SARs and binding mode established in this study will be useful for further lead optimization and pharmacokinetic evaluation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

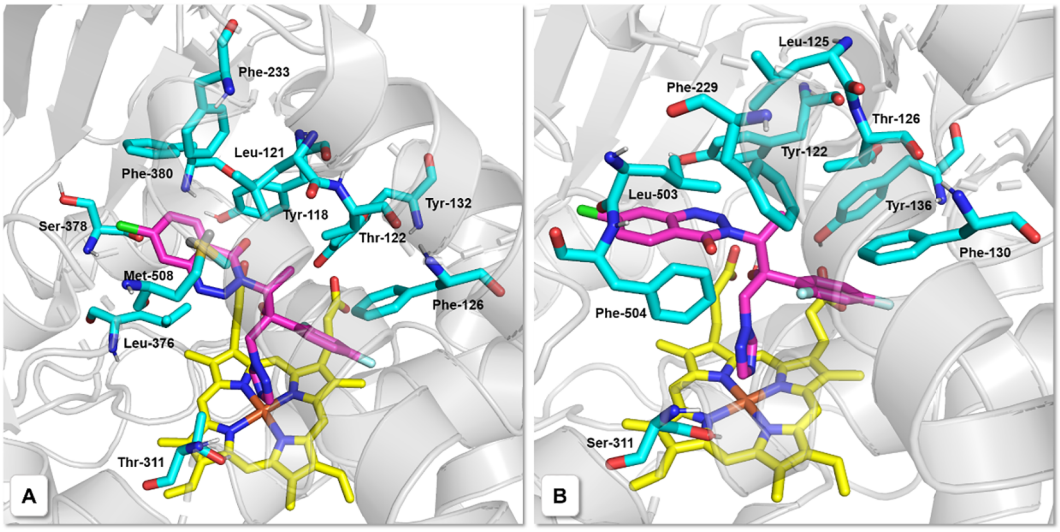

Fig. 3. The binding mode of compound **6m** in the active site of (A) *C. albicans* CYP51 (PDB ID:5TZ1) and (B) *A. fumigatus* CYP51 (PDB ID:4UYM).

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2020.126951.

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