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Original article

New triazole derivatives containing substituted 1,2,3-triazole side chains: design, synthesis and antifungal activity

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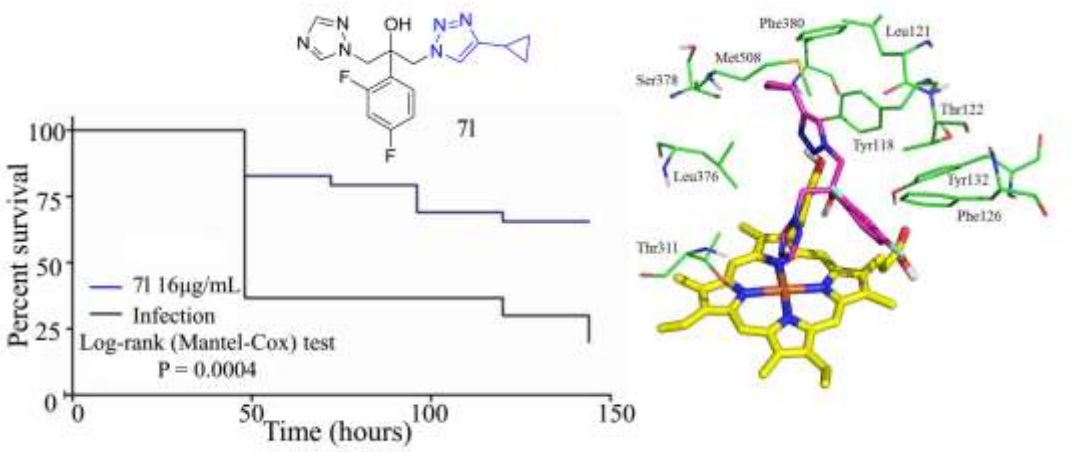
Graphical Abstract



A series of new triazole antifungal derivatives were designed and synthesized. Compound **7l** showed potent in vitro and in vivo antifungal activity.

ABSTRACT

In order to discover new generation of triazole antifungal agents, a series of novel antifungal triazoles were designed and synthesized by structural simplification of our previously identified triazole-piperdine-heterocycle lead compounds. Several target compounds showed good antifungal activity with a broad spectrum. In particular, compound **7l** was highly active against *C. albicans* and *C. glabrata*. Moreover, compound **7l** showed potent *in vivo* antifungal efficacy in the *C. elegans–C. albicans* infection model.

Keywords: Triazole derivatives, Antifungal activities, Click reaction, Molecular Docking, CYP51

1. Introduction

Recently, the incidence of invasive fungal infections (IFIs) and associated mortality has been increasing rapidly mainly due to the large number of immunocompromised patients and limited antifungal agents [1]. Most life-threatening IFIs are caused by *Candida albicans*, *Cryptococcus neoformans* and *Aspergillus fumigatus*, whose mortality rate ranging from 20% to 90% [2, 3]. However, there are only three classes of antifungal agents (*i.e.* polyenes, triazoles and echinocandins) available for the treatment of IFIs [4, 5]. Clinical application of polyene antifungal agent amphotericin B is limited in some severe infections because of serious nephrotoxicity and many other side effects [6, 7]. Echinocandins (*e.g.*, caspofungin and micafungin) have fungicidal activity, but they cannot be orally administrated. Clinically, triazole antifungal agents (*e.g.*, fluconazole, voriconazole, itraconazole, and posaconazole) are widely used as the first-line antifungal therapy for the prevention and treatment of IFIs (Fig. 1). However, broad application of the triazoles has caused severe drug resistance, which significantly reduced the clinical efficacy [8]. Thus, there is still an urgent need for the discovery and development of new generation of triazole antifungal agents [9-13]. For example, isavuconazole [14] was marketed in 2015 for treatment of invasive aspergillosis and invasive mucormycosis and albaconazole [15] are under late stages of clinical evaluations (Fig. 1).

Lanosterol 14α-demethylase (**CYP51**), a key enzyme in fungal membrane ergosterol biosynthesis, is the target of triazole antifungal agents. Due to the difficulties in solving structures of membrane-bound proteins, only one crystal structure of fungal CYP51 (*Saccharomyces cerevisiae* CYP51) has been reported [16]. However, the lack of high-resolution structural information for CYP51 from invasive fungal pathogens limited the structural optimization of the triazoles. Previously, we constructed three-dimensional models of *C. albicans* CYP51 (CACYP51), *C. neoformans* CYP51 (CNCYP51), and *A. fumigatus* CYP51 (AFCYP51) by homology modeling [17-19]. Guided by the binding modes of triazole antifungal agents [17, 20, 21], we rationally designed a number of highly potent new triazoles [21-34]. Among them, triazole **5** showed excellent *in vitro* antifungal activity with a broad antifungal spectrum (Fig. 2). Inspired by the results, further lead optimization was focused on improving the metabolic stability and *in vivo* antifungal

potency. Herein a series of new 1,2,3-triazole containing triazole derivatives were designed and synthesized, which showed potent *in vitro* and *in vivo* antifungal activity.

2. Results and discussion

2.1 Design Rationale and Molecular Docking

In our previous studies, triazole **5** containing a benzyloxypiperidinyl side chain was identified as a potent antifungal agent with a broad spectrum (Fig. 2) [30]. However, it was metabolically unstable because of the benzyl alcohol substructure. Thus, lead compound **5** was further optimized by replacing the benzyl alcohol substructure with substituted heterocycles such as 1,2,3-triazole [31], 1,2,4-oxadiazole and 1,3,4-oxadiazole [34] (Fig. 2). Excellent antifungal activity was retained for these piperidinyl heterocylic derivatives [31, 34]. Inspired by the results, we envisioned that the piperidinyl group can be further removed to reduce molecular weight and increase water solubility. Thus, a series of new traizole derivatives containing substituted 1,2,3-triazole side chains were synthesized and assayed because of its synthetic accessibility and usefulness in antifungal drug discovery [35-37].

In order to investigate whether the designed triazoles can bind well with the active site of CACYP51, the binding mode of compound **7l** was explored by molecular docking [31, 34]. As shown in Fig. 3, the interactions between compound **7l** and CYP51 are similar to those observed in our previous studies [34]. The triazole ring formed a coordination bond with the Fe atom of the heme group and the difluorophenyl group was located into a hydrophobic pocket lined with Phe126 and Tyr132. The 1,2,3-triazole ring formed π-π interaction with Tyr118. Finally, the terminal cyclopropyl group interacted with Leu376, Phe380 through hydrophobic and *Van der Waals* interactions.

2.2 In vitro Antifungal Activity

The inhibitory activity of the target compounds against clinically important pathogenic fungi was determined according to the protocols from National Committee for Clinical Laboratory Standards (NCCLS).The results revealed that most compounds generally showed moderate to excellent activity against the tested fungal pathogens (Table1). Particularly, compounds **7f** (MIC = 0.125μg/mL) and **7l** (MIC = 0.125 μg/mL) were highly active against *C.albicans*, which were more active than fluconazole (MIC = 0.5 μg/mL). In contrast, the target compounds generally showed improved activity against *C.glabrata*, whereas they were less potent against *C. parapsilosis*. For example, the activity of compounds **7j**, **7k**, **7l**, **8a**and **8b** (MIC range: 0.125 ~ 0.5 μg/mL) against *C. glabrata* were comparable or superior to that of fluconazole (MIC = 0.25 μg/mL). For *C. neoformans*, most compounds showed moderate activity except compound **8a** (MIC = 0.25 μg/mL), which was 8 fold more potent than fluconazole (MIC = 2 μg/mL). However, all the compounds as well as fluconazole were inactive against *A. fumigatus* (MIC > 64 μg/mL). For dematophytes (*T. rubrum* and *M. gypseum*), the target compounds also showed moderate to good activity. In particular, compound **7d** (MIC = 0.25 μg/mL) revealed better activity against *T. rubrum* than fluzonazole (MIC = 0.5 μg/mL).

2.3 Structure-activity Relationship

On the basis of the antifungal activity, preliminary structure-activity relationships (SARs) were obtained. For the phenyl derivatives (**7a-f**), 3-substitutions (**7d**, **7e** and **7f**) were more favorable than the 4-substitutions (**7b**, **7c**). Among the 3-substituted derivatives, 3-bromo substitution (**7f**) was more active than the 3-methyl substitution (**7d**). However, 3-chloro substitution (**7e**) had little effect on the antifungal activity as compared to the unsubstituted compound **7a**. When the phenyl group of compound **7a** was replaced by elctron-rich thiophene (**7h**, **7i**), the antifungal activity was significantly improved. In contrast, electron-deficient pridinyl group (**7g**) led to substantial decrease of the antifungal activity. Replacement of the phenyl group of compound **7a** by acycloalkyl group, namely cyclopentyl (**7i**), cyclohexyl (**7j**) and cyclopropyl (**7k**), resulted in the improvement of antifungal activity. In contrast, compound with the *tert*-butyl substitution (**7m**) only showed moderate activity. Good antifungal activity was retained when the substitution was attached on the N1 position of 1,2,3-triazole (**8a** and **8b**).

*2.4 In vivo antifungal activity of compound **7l***

Finally, the *in vivo* antifungal activity of compound **7l** was evaluated in a *Caenorhabditis elegans–C. albicans* infection model [38]. Nematodes were infected with *C. albicans* for 4 h and then moved to pathogen-free liquid medium in the presence of compound **7l**, fluconazole or DMSO. Dead worms were counted and removed daily. *C. elegans'* survival was examined by using the Kaplan-Meier method and differences were determined by using the log-rank test. Interestingly, compound **7l** showed potent *in vivo* antifungal efficacy, which could effectively protect *C. elegans* from *C. albicans* infection (Fig. 4A). At the concentration of 16 μg/mL, *C. elegans* survival rate of compound **7l** was about 70%, which was higher than that of fluconazole (60% survival rate at the concentration of 32 μg/mL, Fig. 4B).

3. Conclusion

In summary, a series of newtriazole antifungal derivatives were designed and synthesized by structural simplification of the triazole-piperdine lead compound. Several target compounds were highly active against a variety of fungal pathogens. In particular, compound **7l** showed potent *in vitro* and *in vivo* antifungal activity, which can serve as a good lead compound for further optimization.

4. Experimental

Nuclear magnetic resonance (NMR) spectra were generated on a Bruker AVANCE300 and AVANCE500 spectrometer (Bruker Company, Germany), using $CDCl_3$ as the reference standard or DMSO-d_6. Chemical shifts (δ values) and coupling constants (J values) are expressed in ppm and Hz, respectively. ESI mass spectra were gathered on an API-3000 LC-MS spectrometer. High-resolution mass spectrometry data were collected on a Kratos Concept mass spectrometer. TLC analysis was carried out on silica gel plates GF254 (Qindao Haiyang Chemical, China). Silica gel column chromatography was performed with Silica gel 60 G (Qindao Haiyang Chemical, China). Commercial solvents were used without any pretreatment.

The synthetic route of the target compounds is outlined in Scheme 1. The ring-open reaction between oxirane intermediate **9** [21] and NaN_3 afforded the azide compound **10.** Then, target compounds **7a-m** was synthesized by click reaction of intermediate **10** with various alkynes in H_2O/^{t}BuOH in the presence of $CuSO_4$ and sodium ascorbate. Propargylation of ketone **11** by propargyl bromide afforded intermediate **12**, which reacted with RN_3 to give target compounds **8a-b** using a similar click reaction procedure.

*4.1 Synthesis of 2-(2,4-difluorophenyl)-1-(4-phenyl-1H-1,2,3-triazol-1-yl)-3-(1H-1,2,4-triazol-1-yl)propan-2-ol (**7a**)*

To a solution of compound **10** (200 mg, 0.71 mmol, 1 equiv) and phenylacetylene (72.0 mg, 0.71 mmol, 1 equiv) in the mixed solvent *tert*-butyl alcohol/H_2O (5:1, 24 mL) was added a mixture of $CuSO_4$ aqueous solution (0.005 mol/L, 1.4 mL) and sodium ascorbate aqueous solution (0.01 mol/L, 7.1 mL). The reaction mixture was stirred at room temperature overnight under the N_2 atmophere. Then, *tert*-butyl alcohol was removed under reduced pressure and the residue was diluted with CH_2Cl_2 (50 mL) and washed by saturated saline (20 mL×3). The organic layers were dried over Na_2SO_4, filtrated and evaporated under reduced pressure. The crude product was purified by chromatography using CH_2Cl_2/MeOH as eluents (50:1 - 30:1) to give target compound **7a** as a white solid: 150 mg, yield 56%. ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.10 (s, 1H), 7.88 (s, 1H), 7.85 (s, 1H), 7.80-7.75 (m, 2H), 7.49-7.43 (m, 1H), 7.41 (t, 2H, J = 7.6 Hz), 7.34 (t, 1H, J = 7.4 Hz), 6.86-6.80 (m, 1H), 6.80-6.75 (m, 1H), 4.91 (dd, 2H, J = 20.2, 14.4 Hz), 4.78 (d, 1H, J = 14.4 Hz), 4.39 (d, 1H, J = 14.3 Hz). ^{13}C NMR (151 MHz, $CDCl_3$): δ 164.07 (d, J = 12.2 Hz), 162.40 (d, J = 12.5 Hz), 159.41 (d, J = 11.6 Hz), 157.77 (d, J = 11.6 Hz), 151.70 (s), 147.73 (s), 144.56 (s), 130.11 (s), 128.89 (s), 128.39 (s), 125.73 (s), 121.81 (s), 112.22 (d, J = 18.8 Hz), 104.43 (t, J = 26.5 Hz), 75.33 (d, J = 4.4 Hz), 56.04 (s), 54.65 (s). MS (ESI) *m/z*: 383.92 (M+H). HR-MSESI$^+$: $[M + H]^+$ calcd. for $C_{19}H_{17}F_2N_6O$, 383.2411; found, 383.2411.

*4.2 The synthetic procedure for compounds **7b-m** and **8a-b** was similar to the synthesis of compound **7a**.*

2-(2,4-Difluorophenyl)-1-(4-(4-fluorophenyl)-1*H*-1,2,3-triazol-1-yl)-3-(1*H*-1,2,4-triazol-1-yl)propan-2-ol **(7b)**: ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.02 (s, 1H), 7.86 (s, 1H), 7.84 (s, 1H), 7.76 (m, 2H), 7.45 (m, 1H), 7.11 (t, 1H, J = 8.6 Hz), 6.80 (m, 2H), 5.51 (brs, 1H), 4.94 (d, 1H, J = 14.3 Hz), 4.90 (d, 1H, J = 14.3 Hz), 4.74 (d, 1H, J = 14.3 Hz), 4.32 (d, 1H, J = 14.3 Hz). MS (ESI) *m/z*: 401.25 (M+H)

2-(2,4-Difluorophenyl)-1-(4-(p-tolyl)-1*H*-1,2,3-triazol-1-yl)-3-(1*H*-1,2,4-triazol-1-yl)propan-2-ol **(7c)**: ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.01 (s, 1H), 7.84 (s, 2H), 7.67 (d, 2H, J = 7.9 Hz), 7.45 (m, 1H), 7.22 (d, 2H, J = 7.9 Hz), 6.79 (m, 2H), 5.49 (brs, 1H), 4.91 (d, 1H, J = 14.4 Hz), 4.87 (d, 1H, J = 14.4 Hz), 4.74 (d, 1H, J = 14.3 Hz), 4.32 (d, 1H, J = 14.4 Hz). MS (ESI) *m/z*: 397.38 (M+H).

2-(2,4-Difluorophenyl)-1-(4-(m-tolyl)-1*H*-1,2,3-triazol-1-yl)-3-(1*H*-1,2,4-triazol-1-yl)propan-2-ol **(7d)**: ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.03 (s, 1H), 7.86 (s, 1H), 7.84 (s, 1H), 7.64 (s, 1H), 7.56 (d, 1H, J = 7.6 Hz), 7.45 (m, 1H), 7.30 (t, 1H, J = 7.6 Hz), 7.15 (d, 1H, J = 7.4 Hz), 6.79 (m, 2H), 5.49 (brs, 1H), 4.92 (d, 1H, J = 14.0 Hz), 4.87 (d, 1H, J = 14.0 Hz), 4.75 (d, 1H, J = 14.0 Hz), 4.32 (d, 1H, J = 14.0 Hz). ^{13}C NMR (151 MHz, $CDCl_3$): δ 164.07 (d, J = 12.5 Hz), 162.40 (d, J = 12.5 Hz), 159.38 (d, J = 11.8 Hz), 157.75 (d, J = 12.0 Hz), 152.15 (s), 147.88 (s), 138.60 (s), 130.16 (dd, J = 9.3, 5.0 Hz), 130.01 (s), 129.15 (s), 128.78 (s), 126.41 (s), 122.83 (s), 121.75 (s), 112.24 (d, J = 18.8 Hz), 104.42 (t, J = 26.5 Hz), 75.41 (d, J = 4.1 Hz), 56.02 (d, J = 3.6 Hz), 54.65 (d, J = 2.3 Hz), 21.41 (s). MS (ESI) *m/z*: 397.34 (M+H). HRMS-ESI$^+$: $[M + H]^+$ calcd. for $C_{20}H_{19}F_2N_6O$, 397.3658; found, 397.3658.

1-(4-(3-Chlorophenyl)-1*H*-1,2,3-triazol-1-yl)-2-(2,4-difluorophenyl)-3-(1*H*-1,2,4-triazol-1-yl)propan-2-ol **(7e)**: ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.09 (s, 1H), 7.94 (s, 1H), 7.87 (s, 1H), 7.80 (t, 1H, J = 1.7 Hz), 7.69 (dt, 1H, J = 7.6, 1.3 Hz), 7.46 (td, 1H, J = 9.0, 6.4 Hz), 7.36 (t, 1H, J = 7.8 Hz), 7.33-7.30 (m, 1H), 6.86-6.82 (m, 1H), 6.81-6.77 (m, 1H), 4.96 (d, 1H, J = 14.4 Hz), 4.92 (d, 1H, J = 14.3 Hz), 4.77 (d, 1H, J = 14.4 Hz), 4.35 (d, 1H, J = 14.3 Hz). ^{13}C NMR (151 MHz, $CDCl_3$): δ 164.12 (d, J = 12.5 Hz), 162.45 (d, J = 12.5 Hz), 159.35 (d, J = 11.9 Hz), 157.72 (d, J = 11.7 Hz), 151.81 (s), 146.52 (s), 144.47 (s), 134.88 (s), 131.97 (s), 130.18 (s), 128.34 (s), 125.82 (s), 123.79 (s), 122.17 (s), 112.31 (d, J = 20.7 Hz), 104.48 (t, J = 26.5 Hz), 75.38 (d, J = 4.4 Hz), 56.07 (d, J = 3.7 Hz), 54.50 (d, J = 5.6 Hz). MS (ESI) m/z: 417.47 (M+H). HRMS-ESI$^+$: $[M + H]^+$ calcd. for $C_{19}H_{16}ClF_2N_6O$, 417.1023; found, 417.1023.

1-(4-(3-Bromophenyl)-1*H*-1,2,3-triazol-1-yl)-2-(2,4-difluorophenyl)-3-(1*H*-1,2,4-triazol-1-yl)propan-2-ol **(7f)**: ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.05 (s, 1H), 7.94 (s, 1H), 7.93 (s, 1H), 7.86 (s, 1H), 7.73 (d, 1H, J = 7.6 Hz), 7.45 (m, 2H), 7.29 (t, 1H, J = 7.6 Hz), 6.80 (m, 2H), 5.51 (brs, 1H), 4.95 (d, 1H, J = 14.2 Hz), 4.95 (d, 1H, J = 14.2 Hz), 4.90 (d, 1H, J = 14.0 Hz), 4.75 (d, 1H, J = 14.0 Hz), 4.32 (d, 1H, J = 14.0 Hz). MS (ESI) *m/z*: 461.24 (M+H).

2-(2,4-Difluorophenyl)-1-(4-(pyridin-3-yl)-1*H*-1,2,3-triazol-1-yl)-3-(1*H*-1,2,4-triazol-1-yl)propan-2-ol **(7g)**: ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.97 (s, 1H), 8.57 (d, 1H, J = 4.2 Hz), 8.17 (d, 1H, J = 7.9 Hz), 8.02 (s, 1H), 7.98 (s, 1H), 7.84 (s, 1H), 7.46 (m, 1H), 7.37 (dd, 1H, J = 5.0, 7.8 Hz), 6.82 (m, 2H), 5.58 (brs, 1H), 4.95 (d, 1H, J = 14.4 Hz), 4.93 (d, 1H, J = 14.4 Hz), 4.77 (d, 1H, J = 14.4 Hz), 4.31 (d, 1H, J = 14.4 Hz). MS (ESI) *m/z*: 384.22 (M+H).

2-(2,4-Difluorophenyl)-1-(4-(thiophen-2-yl)-1*H*-1,2,3-triazol-1-yl)-3-(1*H*-1,2,4-triazol-1-yl)propan-2-ol **(7h)**: ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.00 (s, 1H), 7.83 (s, 1H), 7.81 (s, 1H), 7.44(m, 1H), 7.35 (d, 1H, J = 3.3 Hz), 7.29 (d, 1H, J = 5.0 Hz), 7.06 (dd, 1H, J = 3.3, 5.0 Hz), 6.80 (m, 2H), 5.50 (brs, 1H), 4.92 (d, 1H, J = 14.5 Hz), 4.87 (d, 1H, J = 14.5 Hz), 4.71 (d, 1H, J = 14.5 Hz), 4.31 (d, 1H, J = 14.5 Hz). MS (ESI) *m/z*: 389.38 (M+H).

2-(2,4-Difluorophenyl)-1-(4-(thiophen-3-yl)-1*H*-1,2,3-triazol-1-yl)-3-(1*H*-1,2,4-triazol-1-yl)propan-2-ol **(7i)**: ^{1}H NMR (600 MHz, DMSO-d_6): δ 8.39 (s, 1H), 8.23 (s, 1H), 7.86 (s, 1H), 7.83-7.80 (m, 1H), 7.63 (dd, 1H, *J* = 4.9, 3.0 Hz), 7.48 (dd, 1H, *J* = 5.0, 1.0 Hz), 7.27 (t, 1H, *J* = 10.5 Hz), 7.21 (dd, 1H, *J* = 15.9, 8.8 Hz), 6.89 (t, 1H, *J* = 8.4 Hz), 6.56 (s, 1H), 5.03 (d, 1H, *J* = 14.4 Hz), 4.80 (d, 1H, *J* = 14.5 Hz), 4.72 (d, 1H, *J* = 14.4 Hz), 4.66 (d, 1H, *J* = 14.5 Hz). ^{13}C NMR (151 MHz, DMSO-d_6): δ 163.31 (d, *J* = 12.3 Hz), 161.68 (d, *J* = 12.6 Hz), 160.26 (d, *J* = 12.4 Hz), 158.62 (d, *J* = 12.1 Hz), 151.32 (s), 145.66 (s), 142.72 (s), 132.35 (s), 130.21 (dd, *J* = 9.0, 5.8 Hz), 127.41 (s), 126.14 (s), 122.72 (s), 121.11 (s), 111.36 (d, *J* = 20.8 Hz), 104.45 (t, *J* = 26.9 Hz), 74.27 (d, *J* = 4.5 Hz), 55.95 (d, *J* = 3.9 Hz), 55.26 (d, *J* = 4.5 Hz). MS (ESI) m/z: 389.27 (M+H). HRMS-ESI$^+$: [M + H]$^+$ calcd. for $C_{17}H_{15}F_2N_6OS$, 389.1066; found, 389.1066.

1-(4-Cyclopentyl-1*H*-1,2,3-triazol-1-yl)-2-(2,4-difluorophenyl)-3-(1*H*-1,2,4-triazol-1-yl)propan-2-ol **(7j)**: ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.06 (s, 1H), 7.84 (s, 1H), 7.43 (m, 1H), 7.30 (s, 1H), 6.80 (m, 2H), 5.41 (brs, 1H), 4.85 (d, 1H, *J* = 14.0 Hz), 4.75 (d, 1H, *J* = 14.4 Hz), 4.68 (d, 1H, *J* = 14.4 Hz), 4.35 (d, 1H, *J* = 14.0 Hz), 3.12 (m, 1H), 2.04 (m, 2H), 1.65 (m, 6H). ^{13}C NMR (151 MHz, $CDCl_3$): δ 164.01 (d, *J* = 12.6 Hz), 162.35 (d, *J* = 12.3 Hz), 159.38 (d, *J* = 11.8 Hz), 157.75 (d, *J* = 11.8 Hz), 152.74 (s), 151.93 (s), 130.17 (dd, *J* = 9.2, 5.3 Hz), 121.88 (s), 112.07 (d, *J* = 21.0 Hz), 104.27 (t, *J* = 26.5 Hz), 75.39 (d, *J* = 3.9 Hz), 55.82 (d, *J* = 3.5 Hz), 54.67 (d, *J* = 2.6 Hz), 36.55 (s), 33.12 (d, *J* = 9.4 Hz), 25.04 (s). MS (ESI) m/z: 375.30 (M+H). HRMS-ESI$^+$: [M + H]$^+$ calcd. for $C_{18}H_{21}F_2N_6O$, 375.1745; found, 375.1745.

1-(4-Cyclohexyl-1*H*-1,2,3-triazol-1-yl)-2-(2,4-difluorophenyl)-3-(1*H*-1,2,4-triazol-1-yl)propan-2-ol **(7k)**: ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.05 (s, 1H), 7.81 (s, 1H), 7.39 (dd, 1H, *J* = 15.5, 8.9 Hz), 6.81-6.72 (m, 2H), 5.47 (s, 1H), 4.83 (d, 1H, *J* = 14.4 Hz), 4.71 (q, 2H, *J* = 14.3 Hz), 4.35 (d, 1H, *J* = 14.3 Hz), 2.70-2.64 (m, 1H), 1.94 (t, 2H, *J* = 11.0 Hz), 1.74 (d, 2H, *J* = 12.5 Hz), 1.68 (d, 1H, *J* = 13.3 Hz), 1.40-1.26 (m, 4H), 1.25-1.19 (m, 1H). ^{13}C NMR (151 MHz, $CDCl_3$): δ 162.33 (d, *J* = 12.6 Hz), 159.37 (d, *J* = 12.0 Hz), 157.78 (s), 156.23 (s), 153.62 (s), 151.81 (s), 144.61 (s), 130.16 (dd, *J* = 9.4, 5.5 Hz), 121.61 (s), 112.07 (d, *J* = 18.7 Hz), 104.27 (t, *J* = 26.2 Hz), 75.37 (d, *J* = 4.6 Hz), 55.82 (d, *J* = 4.1 Hz), 54.65 (d, *J* = 5.6 Hz), 35.02 (s), 32.86 (s), 25.97 (s). HRMS-ESI$^+$: [M + H]$^+$ calcd. for $C_{19}H_{23}F_2N_6O$, 389.1909; found, 389.1909.

1-(4-Cyclopropyl-1*H*-1,2,3-triazol-1-yl)-2-(2,4-difluorophenyl)-3-(1*H*-1,2,4-triazol-1-yl)propan-2-ol **(7l)**: ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.09 (s, 1H), 7.85 (s, 1H), 7.44 (dt, 1H, *J* = 15.3, 7.7 Hz), 7.32 (s, 1H), 6.81 (tdd, 2H, *J* = 10.9, 8.3, 2.3 Hz), 4.87 (d, 1H, *J* = 14.3 Hz), 4.78 (d, 1H, *J* = 14.3 Hz), 4.66 (d, 1H, *J* = 14.3 Hz), 4.33 (d, 1H, *J* = 14.2 Hz), 1.91 (ddd, 1H, *J* = 17.0, 8.5, 5.0 Hz), 0.94 (dd, 2H, *J* = 8.4, 2.2 Hz), 0.81-0.77 (m, 2H). ^{13}C NMR (151 MHz, $CDCl_3$): δ 164.04 (d, *J* = 12.1 Hz), 162.37 (d, *J* = 12.3 Hz), 159.36 (d, *J* = 12.0 Hz), 157.73 (d, *J* = 11.6 Hz), 151.72 (s), 150.26 (s), 130.17 (dd, *J* = 9.3, 5.3 Hz), 121.95 (s), 112.17 (d, *J* = 20.8 Hz), 104.35 (t, *J* = 26.5 Hz), 75.33 (d, *J* = 4.5 Hz), 55.81 (d, *J* = 4.0 Hz), 54.64 (d, *J* = 5.2 Hz), 7.80 (d, *J* = 10.7 Hz), 6.52 (s). MS (ESI) *m/z*: 347.22 (M+H). HRMS-ESI$^+$: [M + H]$^+$ calcd. for $C_{16}H_{17}F_2N_6O$, 347.1498; found, 347.1498.

1-(4-(tert-Butyl)-1*H*-1,2,3-triazol-1-yl)-2-(2,4-difluorophenyl)-3-(1*H*-1,2,4-triazol-1-yl)propan-2-ol **(7m)**: ^{1}H NMR (600 MHz, DMSO-d_6): δ 8.36 (s, 1H), 7.84 (s, 1H), 7.54 (s, 1H), 7.20 (dt, 2H, *J* = 16.0, 10.1 Hz), 6.89 (t, 1H, *J* = 8.5 Hz), 6.43 (s, 1H), 4.89 (d, 1H, *J* = 14.3 Hz), 4.78 (d, 1H, *J* = 14.4 Hz), 4.64 (d, 1H, *J* = 14.4 Hz), 4.59 (d, 1H, *J* = 14.5 Hz), 1.20 (s, 9H). ^{13}C NMR (151 MHz, DMSO-d_6): δ 163.36 (d, *J* = 12.7 Hz), 161.73 (d, *J* = 12.7 Hz), 160.26 (d, *J* = 12.5 Hz), 158.62 (d, *J* = 12.4 Hz), 156.10 (s), 151.34 (s), 145.65 (s), 130.34 (dd, *J* = 9.2, 5.8 Hz), 121.24 (s), 111.28 (d, *J* = 21.0 Hz), 104.40 (t, *J* = 26.9 Hz), 74.43 (d, *J* = 4.5 Hz), 55.87 (d, *J* = 3.5 Hz), 55.39 (d, *J* = 4.9 Hz), 30.62 (s). MS (ESI) *m/z*: 363.26 (M+H). HRMS-ESI$^+$: [M + H]$^+$ calcd. for $C_{17}H_{21}F_2N_6O$, 363.1788; found, 363.1788.

1-(1-Cyclopentyl-1H-1,2,3-triazol-4-yl)-2-(2,4-difluorophenyl)-3-(1H-1,2,4-triazol-1-yl)propan-2-ol **(8a)**: ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.15 (s, 1H), 7.80 (s, 1H), 7.39 (m, 1H), 7.18 (s, 1H), 6.72 (m, 2H), 5.54 (brs, 1H), 4.80 (m, 1H), 4.71 (d, 1H, *J* = 14.4 Hz), 4.58 (d, 1H, *J* = 14.3 Hz), 3.15 (d, 1H, *J* = 14.9 Hz), 3.15 (d, 1H, *J* = 14.9 Hz), 2.17 (m, 2H), 1.68-1.95 (m, 6H). ^{13}C NMR (151 MHz, $CDCl_3$): δ 163.41 (d, *J* = 12.2 Hz), 161.76 (d, *J* = 12.2 Hz), 159.38 (d, *J* = 11.7 Hz), 157.74 (d, *J* = 11.8 Hz), 151.27 (s), 142.45 (s), 130.19 (dd, *J* = 9.1, 5.8 Hz), 120.95 (s), 111.36 (d, *J* = 22.9 Hz), 103.90 (t, *J* = 26.6 Hz), 75.20 (d, *J* = 4.6 Hz), 61.84 (s), 57.06 (d, *J* = 3.6 Hz), 33.75 (d, *J* = 4.3 Hz), 29.68 (s), 23.93 (s). MS (ESI) *m/z*: 376.00 (M+H). HRMS-ESI$^+$: [M + H]$^+$ calcd. for $C_{18}H_{21}F_2N_6O$, 375.2012; found, 375.2012.

1-(1-Cyclohexyl-1*H*-1,2,3-triazol-4-yl)-2-(2,4-difluorophenyl)-3-(1*H*-1,2,4-triazol-1-yl)propan-2-ol (**8b)**: ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.24 (s, 1H), 7.82 (s, 1H), 7.38 (dd, 1H, *J* = 15.6, 8.9 Hz), 7.19 (s, 1H), 6.76-6.67 (m, 2H), 4.72 (d, 1H, *J* = 14.0 Hz), 4.60 (d, 1H, *J* = 14.4 Hz), 4.30 (tt, 1H, *J* = 11.8, 3.8 Hz), 3.44 (d, 1H, *J* = 14.9 Hz), 3.14 (d, 1H, *J* = 14.9 Hz), 2.07 (dd, 2H, *J* = 23.9, 12.5 Hz), 1.86 (d, 2H, *J* = 13.9 Hz), 1.72 (d, 1H, *J* = 13.3 Hz), 1.67-1.56 (m, 2H), 1.45-1.35 (m, 2H). ^{13}C NMR (151 MHz, $CDCl_3$): δ 163.41 (d, *J* = 12.2 Hz), 161.76 (d, *J* = 12.6 Hz), 159.36 (d, *J* = 11.7 Hz), 157.73 (d, *J* = 11.8 Hz), 142.20 (s), 130.17 (dd, *J* = 9.2, 5.8 Hz), 124.99 (d, *J* = 9.8 Hz), 120.22 (s), 111.37 (d, *J* = 22.8 Hz), 103.90 (t, *J* = 26.6 Hz), 75.19 (d, *J* = 4.5 Hz), 60.09 (s), 57.23 (s), 33.76 (d, J = 4.2 Hz), 33.37 (d, J = 7.1 Hz), 29.69 (s), 25.03 (s). MS (ESI) *m/z*: 389.69 (M+H). HRMS-ESI$^+$: [M + H]$^+$ calcd. for $C_{19}H_{23}F_2N_6O$, 389.1864; found, 389.1864.

4.3 In vitro antifungal activity assay

In vitro antifungal activity was measured according to the protocols from National Committee for Clinical Laboratory Standards (NCCLS). Serial dilution method in 96-well microtest plate was used to determine the minimum inhibitory concentration (MIC) of the target compounds. Tested fungal strains were obtained from the ATCC or clinical isolates. Briefly, the MIC value was defined as the lowest concentration of tested compounds that resulted in a culture with turbidity less than or equal to 80% inhibition when compared with the growth of the control. Tested compounds were dissolved in DMSO serially diluted in growth medium. The yeasts were

incubated at 35 °C and the mold and dermatophytes at 28 °C. Growth MIC was determined at 24 h for *Candida* species, at 72 h for *Cryptococcus neoformans*, and at 7 days for *Aspergillus fumigatus*.

4.4 In vivo antifungal activity assay

C. elegans was first infected by *C. albicans*. Briefly, *C. elegans glp-4; sek-1* adult nematodes were added to the center of *C. albicans* SC5314 lawns on BHI kanamycin (45 μg/mL) agar plates and incubated at 25°C for 4 h to allow infections. Worms were washed four times with sterile M9. Thirty worms were then pipetted into each well of 12-well tissue culture plates (Corning, USA) containing 2 ml of liquid medium (80% M9, 20% BHI) and kanamycin (45 μg/mL). For compounds treatment groups, compound was added at 16 μg/mL. 32 μg/mL FLC treatment group was set as the positive control, and the DMSO solvent group was set as the negative control. Worms were scored daily and dead worms were removed from the assay. Survival was examined by using the Kaplan-Meier method and differences were determined by using the log-rank test (STATA 6; STATA, College Station, TX). A *P* value of, 0.05 was considered statistically significant.

Acknowledgments

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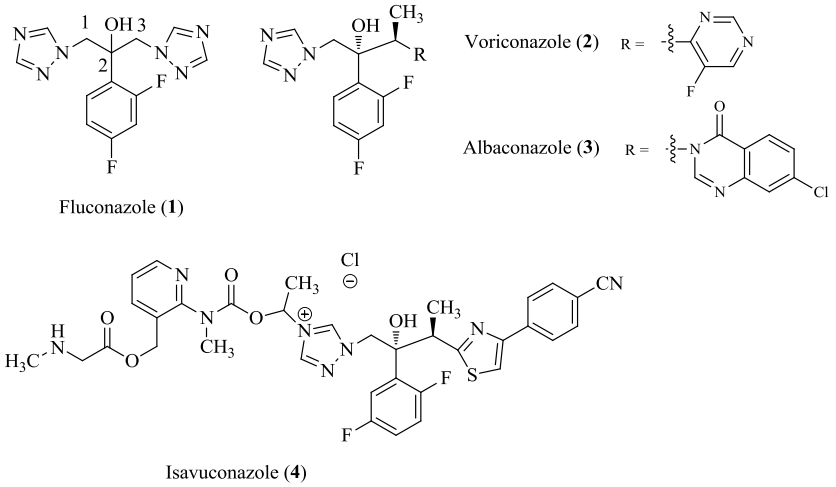


Fig. 1 Structures of triazole antifungal agents

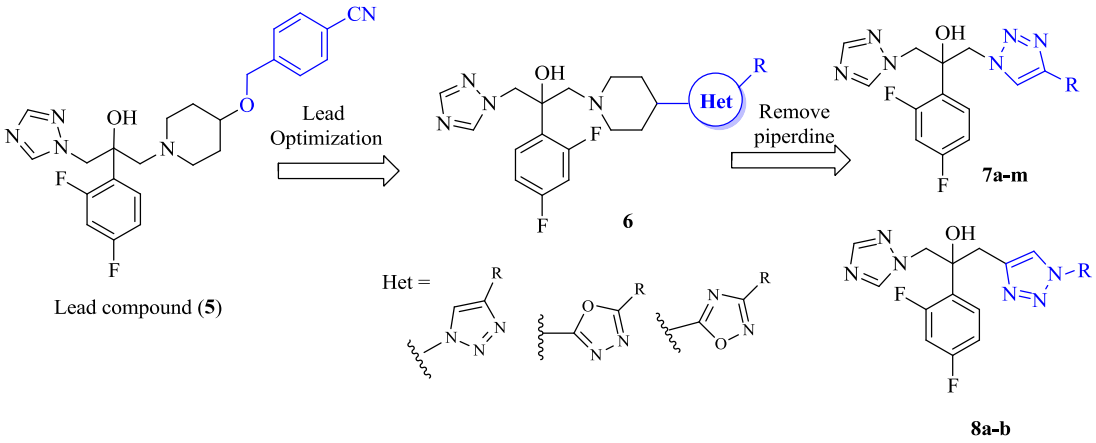


Fig. 2 Design rationale of the target compounds.

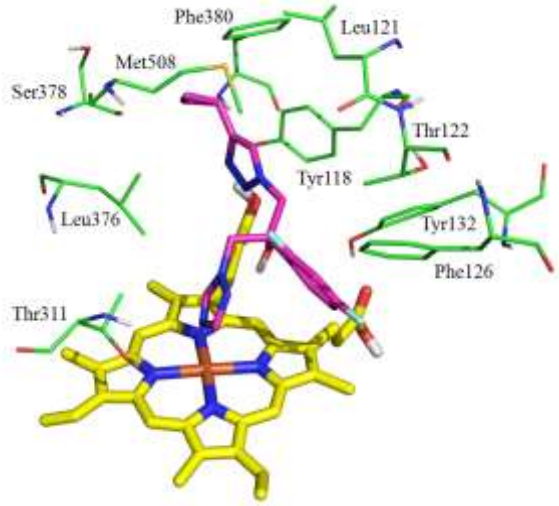


Fig. 3 The binding mode of compound **7l** in the active site of CACYP51.

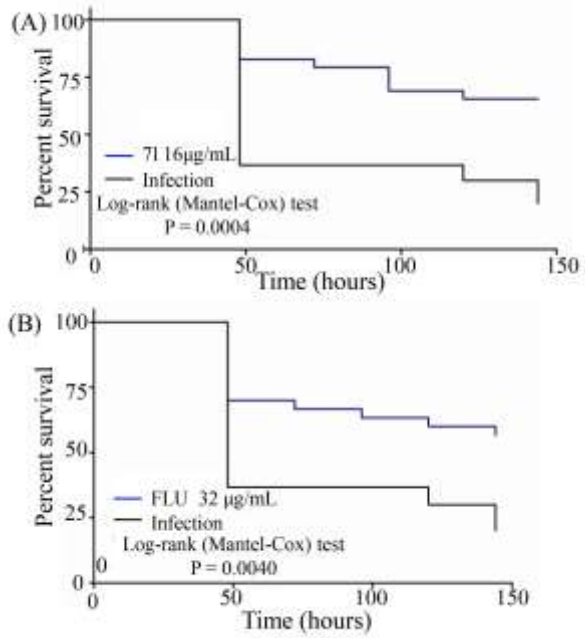
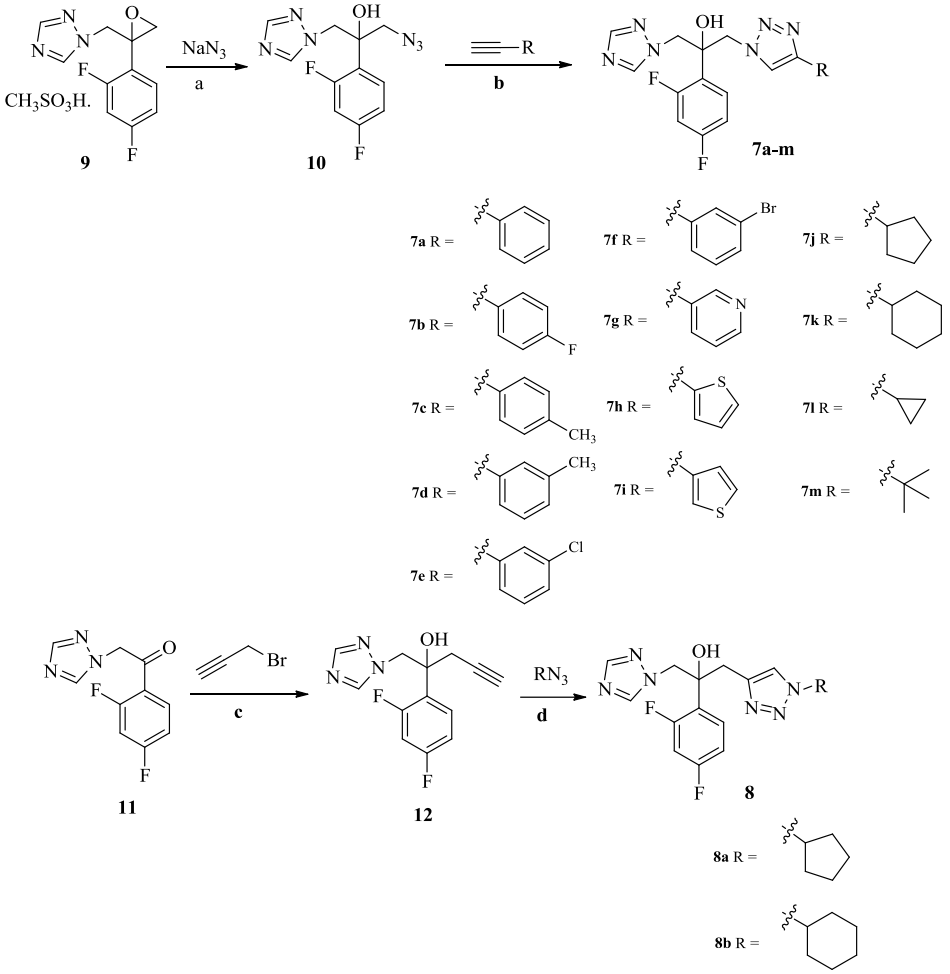


Fig. 4 Compound 7l and fluconazole prolong the survival of *C. elegans glp-4*; *sek-1* nematodes infected by *C. albicans* SC5314. A *P* value of < 0.05 was considered statistically significant.



Scheme 1. (a) NaN_3/NH_4Cl, MeOH, reflux, overnight;(b) $CuSO_4$, sodium ascorbate, *t*-BuOH, H_2O, overnight;(c) Zn, DMF, THF, rt, 5h; (d) $CuSO_4$, sodium ascorbate, H_2O, overnight.

Table 1

In vitro antifungal activities of the target compounds (MIC_{80}, μg/mL)[a]

Compds	*C. alb.*	*C. gla*	*C. para.*	*C. neo.*	*A. fum.*	*T. rub.*	*M. gyp.*
7a	8	0.5	8	8	>64	4	>64
7b	16	8	16	32	>64	8	>64
7c	64	2	64	64	>64	16	>64
7d	0.5	0.5	64	64	>64	0.25	>64
7e	8	0.5	8	8	>64	2	2
7f	0.125	0.5	2	8	>64	8	>64
7g	64	16	64	32	>64	64	>64
7h	0.5	0.5	2	8	>64	2	8
7i	0.5	0.5	4	16	>64	2	4
7j	0.25	0.25	4	8	>64	2	8
7k	0.25	0.25	4	8	>64	2	8
7l	0.125	0.25	2	1	>64	4	8
7m	2	2	32	32	>64	16	>64
8a	1	0.25	1	0.25	>64	2	8
8b	1	0.125	1	1	>64	2	4
FLZ	0.5	0.25	1	2	>64	0.5	2

[a]Abbreviations: *C. alb. Candida albicans; C. gla. Candida glabrata; C. para. Candida parapsilosis; C. neo. Cryptococcus neoformans; A. fum. Aspergillus fumigatus; T. rub. Trichophyton rubrum; M. Gyp. Microsporum gypseum.*