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Research paper

Molecular docking, design, synthesis and antifungal activity study of novel triazole derivatives

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ABSTRACT

The incidence of life-threatening fungal infections has dramatically increased for decades. In order to develop novel antifungal agents, two series of (2*R*,3*R*)-1-(1*H*-1,2,4-triazol-1-yl)-2-(2,4-difluorophenyl)-3-(*N*-substitutied)-2-butanols (**3a-o**, **5a-f**, **8a-u**), which were analogues of voriconazole, were designed, synthesized and characterized by ^{1}H NMR, ^{13}C NMR and HRMS. The MIC_{80} values showed that the target compounds **3a-o** indicated better activities than fluconazole on three important fungal pathogens except for **3i**. Significant activity of compounds **3d**, **3k**, **3n**, **3m** and **3o** was observed on the *Aspergillus fumigatus* strain (MIC_{80} range: 1–0.125 μg/ml). Especially, compound **3k** had strong activity to inhibit the growth of ten fungal pathogens. But it didn't exhibit good activity in *in vivo* value. Molecular docking experiments demonstrated that **3k** possessed superior affinity with target enzyme by strong hydrogen bond from morpholine ring.

1. Introduction

During the last three decades, the increasing morbidity and emergence of drug-resistance in life-threatening fungal infections come to be a significant health problem globally, especially among population with autoimmune diseases, cancer, organ transplants or AIDS [1–5]. Generally, *Candidosis albicans* (*C. alb.*), *cryptococcus neoformans* (*C. neo.*) and *Aspergillus fumigatus* (*A. fum.*) are the major pathogenic agents for systemic fungal infections [6,7]. Azoles (e.g. Fluconazole(FCZ), Itraconazole(ICZ), Voriconazole(VCZ) and Posaconazole(PCZ), Fig. 1) are first-line drugs for mycoses, due to its broad antifungal effect, high potency and low toxicity [8–12].

Noticeably, it is estimated that three million people are suffering from aspergillosis and 450 thousands died per year worldwide [13]. After the first isolation of azole-resistant *A. fum* in 1989, ICZ resistance and cross-resistant to other azoles are becoming a major clinical concern for aspergillosis control currently [13,14]. The antifungal resistant profile of *A. fum* and other pathogenic *aspergillus* undermined the antifungal efficacy and potentially lead to treatment failure [15,16]. Therefore, it is urgent to develop novel antifungal azoles with excellent activity against a variety of clinical fungal pathogens, especially *Aspergillus spp.*

Azoles antifungal act by competitive inhibition of the lanosterol 14α-demethylase (CYP51), the necessary enzyme for catalyzing the oxidative removal of the 14α-methyl in sterol biosynthesis of fungi [17]. Consequently, CYP51 has been regarded as a target for molecular docking and widely applied in the rational design of antifungal compounds [18–24]. To our knowledge, the active site of CYP51 could be divided into four parts [25,26]: (a) a coordination bond with iron of the heme group, (b) the hydrophobic region, (c) the hydrophilic H bonding region, (d) the narrow hydrophobic cleft formed by the residues in the N-terminus of helix I and helix B′-meander 1 loop.

Prior studies had reported pharmacophores of antifungal triazoles [18–24], which containing a linker was formed through a two carbon chain between a triazole ring and a dihalophenyl ring.

Abbreviations: C. alb., *Candidosis albican*; *C. neo.*, *Cryptococcosis neoformans*; *A. fum.*, *Aspergillus fumigatus*; FCZ, Fluconazole; ICZ, Itraconazole; VCZ, Voriconazole; PCZ, Posaconazole; CYP51, lanosterol 14α-demethylase; iPrOH, isopropanol; DMSO, dimethyl sulfoxide; MIC, Minimum inhibitory concentration; *T. rub.*, *Trichophyton rubru*; *M. gyp.*, *Microsporum gypseu.*

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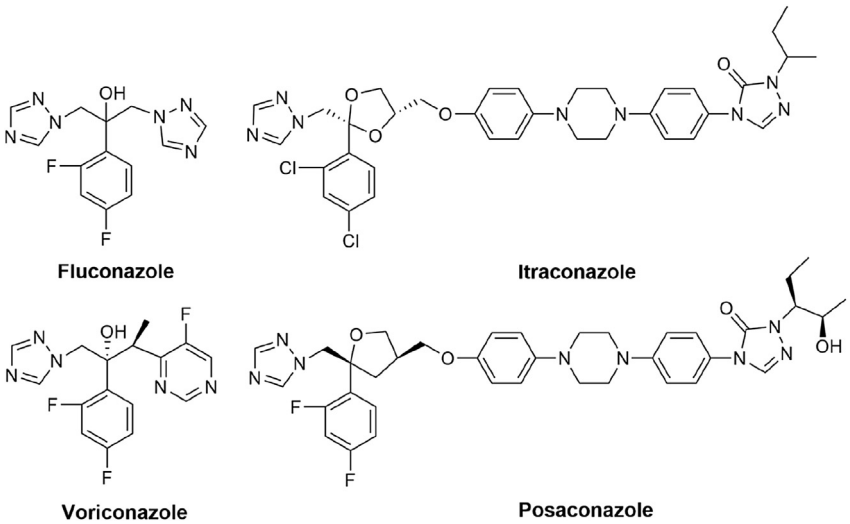

Fig. 1. Triazole antifungal agents used in clinical therapy.

In addition, the carbon alpha to the phenyl ring bored a hydroxyl group. This binding pattern was further examined by our 3D computer modeling of the interaction between VCZ and CYP51 of *A. fum.* according to the Ji et al.'s study [27]. As shown in Fig. 2, the pyrimidine group of VCZ interacted with HIS374, SER375, ILF376, LEU503, PHE504, and ILE373 through the hydrophobic cleft. The residues, including ALA307, HEM503, ALA303, PHE130, VAL135, THR126, TYR136, and TYR122, were further considered as forming nonbonding interactions with the triazolyl group, hydroxyl group and difluorophenyl group.

In order to find effective systemic antifungal compounds with a broad antifungal spectrum and less potential to develop resistance, two new series of (2*R*,3*R*)-1-(1*H*-1,2,4-triazole-1-yl)-2-(2,4-difluorophenyl)-3-(*N*-substitutied)-2-butanols (Fig. 3) were designed. While these compounds included all the essential pharmacophores, the introduction of an additional amine or 1,2,3-triazole group in side chain was expected to potentially result in preferable systemic antifungal compounds that are less possible to develop drug resistance. Amines and 1,2,3-triazoles were considered as privileged building blocks for the synthesis of bioconjugates

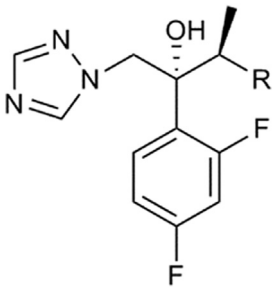

Fig. 3. Generic structure of the designed Voriconazole analogues.

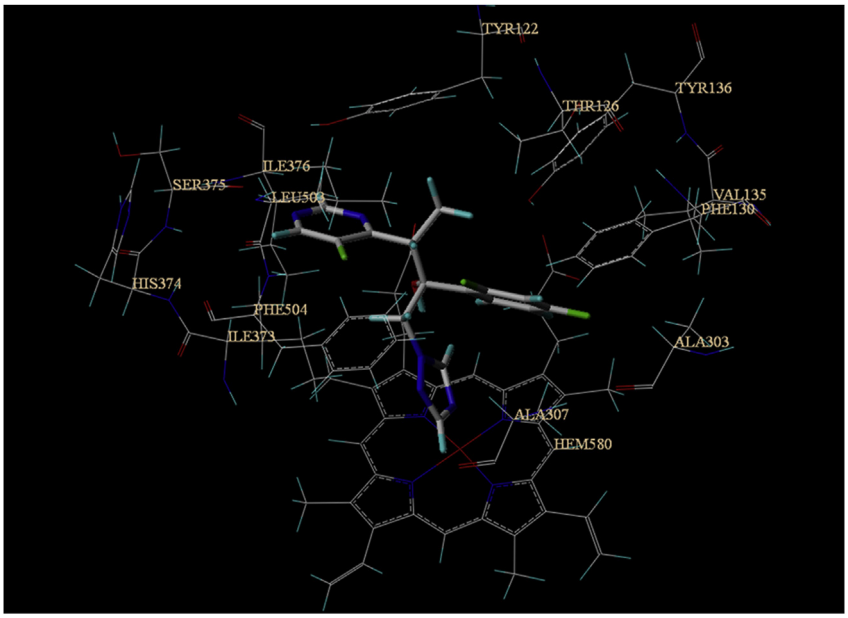

Fig. 2. Computed Voriconazole binding to the heme iron atom at the active site of CYP51.

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due to their high stability, selectivity and less adverse reactions [18,20,28–30]. These structures indicated formidable stable under basic and acid hydrolysis including oxidative and reductive reactions. What's more, we systematically altered the side chain structure which was oriented to interact with the narrow hydrophobic cleft, to study how it may further affect the antifungal activity.

2. Chemistry

In Scheme 1, synthesis of the target compounds **3a-o** were achieved by reacting with intermediate oxirane **1** and primary or secondary amines (**2a-o**) in the presence of $LiClO_4$ in isopropanol (iPrOH) at 120 °C. In order to further investigate the effect of antifungal activity during various linkers, novel azoles **5a-f** were synthesized on the basis of the nucleophilic substitution reaction from potential compound **3d**. Accordingly, compounds **5a-f** were prepared through **3d** reacted with substituted alkyl halides in NaH, KI and Acetonitrile (Scheme 2). In addition, as shown in Scheme 3, the ring-opening reaction of oxirane **1** with NaN_3 to give key intermediate **7**. This was subsequently treated with substituted alkynes to obtain target compounds **8a-u** through Cu-catalyzed azide-alkyne cycloaddition.

3. Pharmacology

3.1. In vitro antifungal activity

According to the National Committee for Clinical Laboratory Standards (NCCLS) recommendations [31,32]. *In vitro* antifungal activity of target compounds **3a-o**, **5a-f** and **8a-u** were measured by means of the minimum inhibitory concentration (MIC). FCZ and VCZ were included in positive controls to compare with target compounds. Tested pathogenic fungi were obtained from the American Type Culture Collection (ATCC). MIC_{80} was defined as the first well with an approximate 80% reduction in growth compared to the growth of the drug-free well. For assays, test compounds and positive controls were dissolved in dimethyl sulfoxide (DMSO) in growth medium, inoculated and incubated at 35 °C. The growth MIC_{80} was determined at 24 h for *C. alb.*, at 72 h for *C. neo.* and at 5–7 days for filamentous fungi. These data were the mean of three replicate tests performed with each test compound and summarized in Table 1 and Table 2.

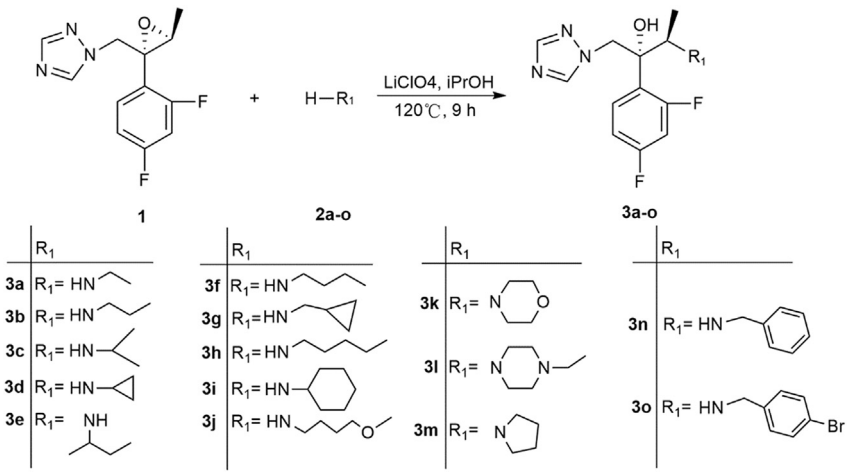
3.2. In vivo antifungal activity

Compound **3k** and FCZ were studied in an infection model of systemic *C. alb.* SC5314 in mice. ICR mice, weighing between 18 and 22 g (male, SLAC Lab Animal Ltd), were used in the model, with each group included ten mice. Firstly, mice were operated intraperitoneal injection of *iv* with 0.4 ml cyclophosphamide (100 mg/kg, 3 days, qd). Then, mice were administered orally *iv* with 0.4 ml of a suspension containing 2×10^9 CFU/ml of *C. alb*. In this model, compound **3k** and FCZ (as a suspension in 0.5% carboxymethylcellulose in distilled water) were administered orally 2 h after infection (1 mg/kg, 7 days, qd). The vehicle control group received only the vehicle. *In vivo* efficacy was determined by the mean of survival days for 20 days after infections. The results were summarized in Fig. 4.

4. Results and discussion

The MIC_{80} values of compounds **3a-o** indicated that most of the compounds were active against three important fungal pathogens and some of them demonstrated higher activities than FCZ and VCZ, especially for *A. fum.* species. The compounds **3d**, **3m**, **3n**, ($MIC_{80} = 1$ μg/ml) **3o** ($MIC_{80} = 0.5$ μg/ml) and **3k** ($MIC_{80} = 0.125$ μg/ml) were better than FCZ ($MIC_{80} > 64$ μg/ml) and **3k** was preceded VCZ ($MIC_{80} = 0.25$ μg/ml) on the *A. fum.* strain. Except for compound **3i**, the MIC_{80} range for *C. alb.* was 0.5 μg/ml to 0.0156 μg/ml, showing that these compounds were comparable or superior to FCZ and VCZ. Significant activity of them was also observed on the *C. neo* strain (MIC_{80} range: 0.5–0.0156 μg/ml). Particularly, both of compounds **3d** and **3k** ($MIC_{80} = 0.0156$ μg/ml) were superior to VCZ ($MIC_{80} = 0.0625$ μg/ml). Two representative compounds **3d** and **3k** presented strong active against several fungal pathogens in Table 2. The compound **3k** was the most active one during the target compounds in particular. On the *C. neo.*, *Trichophyton rubrum (T. rub.)*, *Microsporum gypseu (M. gyp.)* and *A. fum.* strain, the compound **3k** was superior to FCZ and VCZ. It is worth to further evaluation due to its excellent antifungal activity toward azole-resistant clinical isolate.

Moreover, the compounds **5a-f**, the further structural modification of compound **3d**, did not indicate the improvement from **3d**. Compounds **5b** and **5c** showed similar activity with FCZ in three important fungal pathogens. Only compound **5a** demonstrated a better inhibiting effect in *C. alb.* ($MIC_{80} = 0.125$ μg/ml) and *C. neo.* ($MIC_{80} = 0.125$ μg/ml) than FCZ. The lower activity of Compounds



Scheme 1. Synthetic route of the target compounds **3a-o**.

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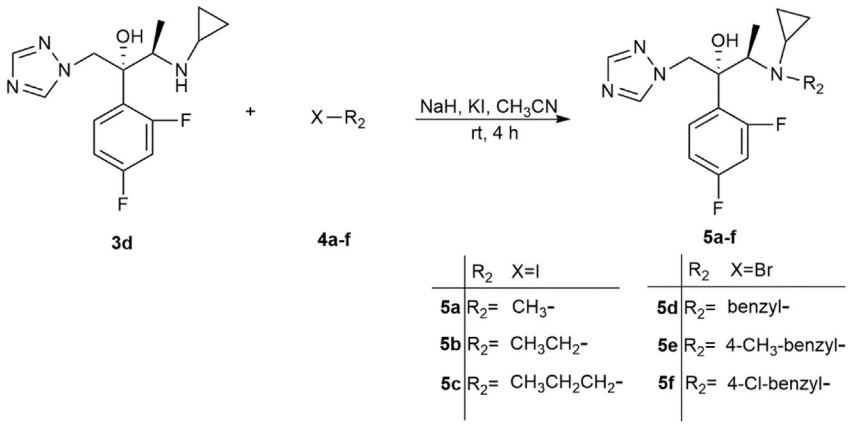
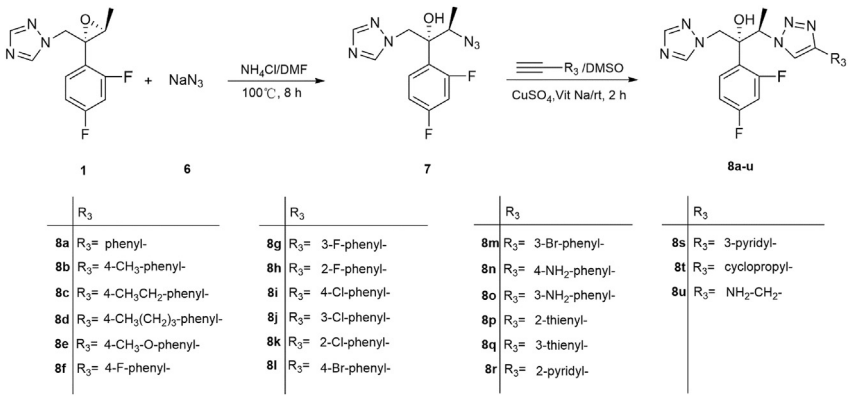


Scheme 2. Synthetic route of the target compounds **5a-f**.



Scheme 3. Synthetic route of the target compounds **8a-u**.

Table 1
Antifungal activities of the target compounds **3a-o**, **5a-f**, **7** and **8a-u** *in vitro*. (MIC = μg/ml).[a]

Compd	*C. abl* SC5314	*C. neo.*	*A. fum.*	*Compd*	*C. abl* SC5314	*C. neo.*	*A. fum.*
3a	0.5	0.5	32	**8a**	0.125	0.0625	16
3b	0.125	0.0625	8	**8b**	0.125	0.0625	>64
3c	0.25	0.25	16	**8c**	0.5	0.5	>64
3d	0.0156	0.0156	1	**8d**	4	8	>64
3e	0.125	0.125	4	**8e**	0.125	0.0625	>64
3f	0.125	0.125	8	**8f**	0.0625	0.0625	32
3g	0.125	0.125	8	**8g**	0.25	0.0625	>64
3h	0.125	0.125	4	**8h**	0.125	0.125	32
3i	0.5	0.5	8	**8i**	0.125	0.0156	32
3j	0.25	0.5	2	**8j**	0.0625	0.0625	>64
3k	0.03125	0.0156	0.125	**8k**	0.0156	0.0156	16
3l	2	2	64	**8l**	0.25	0.25	>64
3m	0.125	0.125	1	**8m**	0.5	1	>64
3n	0.03125	0.125	1	**8n**	0.5	0.5	>64
3o	0.0156	0.125	0.5	**8o**	0.5	1	>64
5a	0.125	0.125	8	**8p**	0.0625	0.0625	16
5b	1	1	>64	**8q**	0.0625	0.0625	4
5c	1	2	8	**8r**	0.5	1	>64
5d	8	8	8	**8s**	1	1	>64
5e	8	5	>64	**8t**	0.0625	0.0625	32
5f	8	4	>64	**8u**	0.25	0.25	>64
				7	0.0156	0.0625	8
FCZ	1	2	>64	FCZ	1	2	>64
VCZ	0.03125	0.03125	0.25	VCZ	0.03125	0.03125	0.25

Abbreviations: C. alb, Candida albicans; C. neo, Cryptococcus neoformans; A. fum, Aspergillus fumigates. FCZ, fluconazole; VCZ, voriconazole.
[a] Minimum inhibitory concentration for 80% inhibition of growth.

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Table 2
Antifungal activities of the target compounds **3d** and **3k** *in vitro*. (MIC = μg/ml).[a]

Compd	*C. abl* SC5314	*C. abl* SCY0109	*C. gla,*	*C.neo.*	*C. kru.*	*A.fum.*	*C. tro.*	*C. par*	*M. gyp*	*T. rub*
3d	0.0156	0.0078	0.0156	0.0156	0.5	1	0.5	0.25	0.125	0.0625
3k	0.03125	0.0156	0.03125	0.0156	0.5	0.125	0.5	0.0625	0.0625	0.0625
FCZ	1	0.25	1	2	32	>64	32	0.5	8	1
VCZ	0.03125	0.0156	0.03125	0.0625	0.25	0.25	0.25	0.03125	0.25	0.125

Abbreviations: C. alb, Candida albicans; C. neo, Cryptococcus neoformans; C. par, Candida parapsilosis; C. tro, Candida tropicalis; C. kru, Candida krusei; C. gla, Candida glabrata; T. rub, Trichophyton rubrum; M. gyp, Microsporum gypseu;. A. fum, Aspergillus fumigates. FCZ, fluconazole; VCZ, voriconazole.
[a] Minimum inhibitory concentration for 80% inhibition of growth.

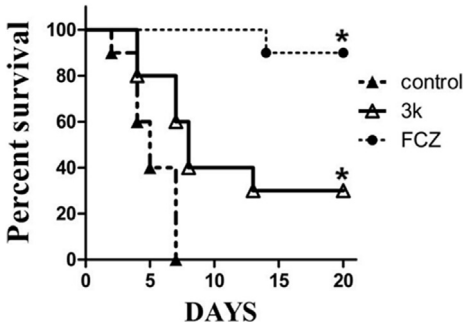

Fig. 4. *In vivo* efficacy of compound **3k** and FCZ (1 mg/kg) in a systemic infection of an ICR mouse model (n = 10) with *C. ald.* SC5314. *, $p < 0.05$.

5a-f probably associated with reduction of the interaction between compounds and target enzyme caused by the extension of molecular structure.

Comparing with the intermediate **7**, establishment of 1,2,3-triazole and introduction of phenyl groups or heterocycle groups on position 4 of the 1,2,3-triazole did not result in a significantly increased antifungal activity. The MIC_{80} range for *C. alb.* and *C. neo.* were 1 μg/ml to 0.0156 μg/ml after excluding **8d**. Furthermore, the various group substituted within this phenyl ring derivatives **8a-o** indicated fluorine and chlorine substituted (**8f-k**) possessed better activity than bromine and amino substituted (**8i-o**). In the other hand, with the extension of carbons in the para-position, antifungal activity became worse (**8a-d**). In addition, the unsubstituted pyridine and thiophene ring analogs (**8p-s**) were tested and proved to be comparable to those of the phenyl analogs. During this novel azoles, compound **8k** was the potent antifungal agent to *C. alb.* and *C. neo.* (MIC_{80} = 0.0156 μg/ml). However, compounds **8a-u** were confirmed as less effective antifungal activity against *A. fum*.

As summarized in Fig. 4, the selected compound **3k**, which demonstrated significant *in vitro* activity as well as broad antifungal spectrum, was evaluated in an ICR mice model infected by *C. alb.* SC5314. The efficacy (survival curve) of orally administered compound **3k** less than that of orally administered FCZ at a dose of 1 mg/kg. Perhaps the lack of inferior activity of compound **3k** could be related to its poor Pharmacokinetics.

On the basis of the molecular docking, we constructed a 3D model of *Aspergillus'* CYP51. A likely binding mode of compound **3k** in the active site of CYP51 was proposed based on docking result (Fig. 5). Sheng et al. found that the interaction between isomers (*R* and *S*) of compound and CYP51 was produced by a similar binding mode. However, *S* isomer showed higher interaction energy with CYP51 than the *R* isomer, which demonstrated that the *R* isomer might had better antifungal activity than the *S* isomer [25]. In our study, the docked conformation refereed to the *R* configuration of the compounds. As shown in Fig. 5, the N(4) atom of the 1,2,4-triazole moiety coordinated with the heme Fe-atom, meanwhile the 2,4-difluorophenyl group in the designed compound could be placed into the hydrophobic pocket formed by ALA303, ALA307, VAL135, PHE130, TYR136 and HEM580. Although the hydroxyl group of compound **3k** did not form direct interaction with CYP51, it might formed hydrogen-bonding interaction with ALA307 mediated by crystal waters. Finally, the morpholine ring could interact with a hydrophobic pocket built by ILE376, PHE504, ILE373, SER375 and HIS374. Especially , the strong hydrogen bond existing between morpholine ring and LEU503 might be the cause of the effective antifungal activity and broad antifungal spectrum. Therefore, the side chain was the key pharmacophore, and the spatial orientations of the pharmacophores were just oriented in the hydrophobic pocket. The introduction of various side chains was a vital factor of novel compounds.

5. Conclusion

In conclusion, two series of novel triazole derivatives with substituted amines or 1,2,3-triazoles as side chain have been synthesized, and their antifungal activities were evaluated for several human pathogenic fungi. *In vitro* biological evaluations of the target compounds **3d** and **3k** showed strong antifungal activities. Novel compound **3k** could against nearly all the tested fungi, particularly, *Aspergillus spp.*. However, the *in vivo* evaluation of the target compound **3k** indicated it possessed inferior activity in the mice. The docking result demonstrated the side chain of morpholine ring could form a closely hydrogen-bonding interaction with CYP51, which may lead to the discovery of a series of compounds for further optimization.

6. Experimental part

Melting points were measured on a Yamato MP-21 melting point apparatus. ^{1}H and ^{13}C Nuclear magnetic resonance (NMR) spectra were achieved in Chloroform-D ($CDCl_3$) unless otherwise indicated with a Bruker AC-300P spectrometer or a Bruker Avance II 600 spectrometer. Tetramethylsilane (TMS) was considered as the internal standard. ESI mass spectra were produced on an Aglient Technologies 6538 UHD Accurate-Mass Q-TOF LC/MS. Silica gel plates GF254 (Yantai Huanghai chemical, china) were applied to thin-layer chromatography (TLC) analysis. Amines were purchased from Energy-Chemical. The solvents and reagents were consumed from commercial vendors and used as received or dried prior to use as needed.

6.1. General procedure for the synthesis of derivatives *3a-o*

To a solution of oxirane **1** (200 mg, 0.796 mmol) in iPrOH (4 ml) were added $LiClO_4$ (170 mg, 1.592 mmol) and **2a** (143 mg, 3.184 mmol). The mixture was stirred continuously for 9 h in an oven-dried pressure-tight reaction tube and heated at 120 °C. The solvent was distilled off under reduced pressure. Then, the crude residue was extracted with dichloromethane (100 ml). The organic layer was washed with H_2O (75 ml × 2) and brine (75 ml × 2), dried

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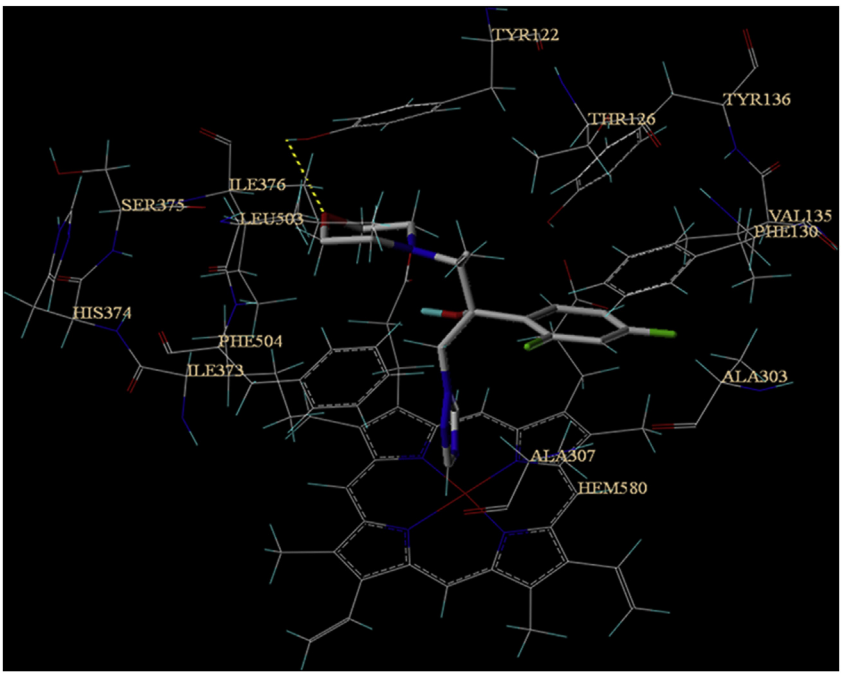

Fig. 5. Computed compound **3k** binding to the heme iron atom at the active site of CYP51.

on anhydrous Na_2SO_4 and filtrated. Then the solvent was evaporated under reduced pressure. The crude residue was purified by flash chromatography on silica gel (PE: EA 10:1–3:1) to compound **3a** as a white solid. Yield: 70.8%; m.p. 100.1–102.9 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.95 (1H, s, triazole-H), 7.74 (1H, s, triazole-H), 7.41–7.37 (1H, m, Ar-H), 6.76–6.73 (2H, s, Ar-H), 4.86–4.72 (2H, m, CH2), 3.08–3.04 (1H, m, –CH), 2.89–2.84 (1H, m, –CH2), 2.60–2.55 (1H, m, –CH2), 1.12–1.09 (3H, m, –CH2), 0.92 (3H, d, J = 6.0 Hz,–CH3); ^{13}C NMR (150 MHz, $CDCl_3$) d: 162.03, 158.23, 150.69, 143.69, 129.89, 123.20, 110.87, 103.28, 56.90, 55.55, 42.12, 29.18, 15.21, 15.05; HRMS (ESI) m/z calcd for $C_{14}H_{18}F_2N_4O$ $[M+H]^+$: 297.1521, found: 297.1523

The target compounds **3b-o** were synthesized by the same procedure as the compound **3a**.

6.2. General procedure for the synthesis of derivatives *5a-f*

The solution of compound **3d** (200 mg, 0.649 mmol) in acetonitrile (5 ml) were added NaH (31.2 mg, 1.298 mmol) and KI (1.6 mg, 0.009 mmol) followed by iodomethane **4a** (184 mg, 1.298 mmol). The reaction was stirred continuously for 4 h at room temperature. The mixture was extracted with dichloromethane (2 × 80 mL), the organic layers were gathered and washed with water (2 × 100 mL) and brine (2 × 100 mL), dried on anhydrous Na_2SO_4, filtered and the solvent was evaporated under reduced pressure. The crude residue was purified by flash chromatography on silica gel (PE: EA 10:1–2:1) to afford compound **5a** as canary yellow solid. Yield: 75.4%; m.p. 119.5–120.9 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.90 (1H, s, triazole-H), 7.75 (1H, s, triazole-H), 7.30–7.25 (1H, m, Ar-H), 6.87–6.78 (2H, s, Ar-H), 5.06–5.01 (1H, m, –CH2), 4.78–4.73 (1H, m, –CH2), 3.36–3.31 (1H, m, –CH), 2.38–2.35 (4H, m, –CH3, –CH), 0.88 (3H, d, J = 6.0 Hz, –CH3), 0.51–0.39 (4H, m, –CH2, –CH2); ^{13}C NMR (75 MHz, $CDCl_3$) d: 164.26, 160.77, 150.64, 144.40, 131.16, 111.35, 104.60, 83.84, 66.36, 54.99, 53.58, 38.59, 37.30, 8.04, 7.64, 6.92; HRMS (ESI) m/z calcd for $C_{16}H_{20}F_2N_4O$ $[M+H]^+$: 323.1678, found: 323.1678

The target compounds **5b-f** were synthesized by the same procedure as the compound **5a**.

6.3. General procedure for the synthesis of derivatives *7*

A mixture of intermediate oxirane **1** (200 mg, 0.796 mmol), NaN_3 (103 mg, 1.260 mmol), NH_4Cl (84 mg, 1.260 mmol) and DMF (10 mL). The reaction mixture was stirred continuously for 8 h in an oven-dried pressure-tight reaction tube and heated at 100 °C. The mixture was extracted with dichloromethane (2 × 80 mL), the organic layers were gathered and washed with water (2 × 100 mL) and brine (2 × 100 mL), dried on anhydrous Na_2SO_4, Then the solvent was evaporated under reduced pressure. The crude residue was purified by flash chromatography on silica gel (PE: EA 10:1–5:1) to intermediate **7** as a white solid. Yield: 90.8%; m.p. 136.7–137.9 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.80–7.75 (2H, m, triazole-H), 7.39–7.33 (1H, m, Ar-H), 6.76–6.69 (2H,m, Ar-H), 4.86–4.72 (2H, m, –CH2), 3.78–3.75 (1H, m, –CH), 1.13 (3H, d, J = 6.0 Hz, –CH3); ^{13}C NMR (75 MHz, $CDCl_3$) d: 162.95, 158.03, 151.93, 143.90, 130.45, 126.460, 111.94, 104.14, 77.80, 59.81, 55.30, 12.98; HRMS (ESI) m/z calcd for $C_{12}H_{12}F_2N_6O$ $[M+H]^+$: 295.1113, found: 295.1112

6.4. General procedure for the synthesis of derivatives *8a-u*

A mixture of intermediate **7** (200 mg, 0.680 mmol), phenylacetylene (128 mg, 1.260 mmol) and DMSO (10 mL). The catalyst of sodium ascorbate (20 mg, 0.100 mmol) and $CuSO_4 \cdot 5H_2O$ (25 mg, 0.100 mmol) in H_2O (1 mL) was added into the reaction solution quickly. The mixture was stirred continuously for 2 h, Then the reaction solution was put into $NH_3 \cdot H_2O$, extracted with dichloromethane (80 mL). The organic layers were gathered and washed with water (2 × 150 mL) and brine (2 × 100 mL), dried on anhydrous Na_2SO_4 and filtered. The solvent was evaporated in a vacuum and crude residue was purified by flash chromatography on silica gel (PE: EA 10:1–2:1) to afford compound **8a** as a white solid. Yield: 92.5%; m.p. 104.2–105.1 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.15 (1H, s, triazole-H), 7.83–7.82 (2H, d, Ar-H), 7.77 (1H, s, triazole-H), 7.66

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(1H, s, triazole-H), 7.46–7.38 (3H, m, Ar-H), 7.31–7.29 (1H, m, Ar-H), 6.79–6.74 (2H, m, Ar-H), 5.48 (1H, s, –OH), 5.46–5.44 (1H, m, –CH), 4.99–4.96 (1H, m, –CH2), 3.62–3.59 (1H, m, –CH2), 1.36 (3H, d, J = 12.0 Hz,–CH3); ^{13}C NMR (150 MHz, $CDCl_3$) d: 163.07, 158.16, 151.85, 147.95, 144.09, 130.55, 130.40, 128.88, 128.88, 128.31, 125.68, 125.68, 122.20, 119.80, 112.05, 104.42, 77.44, 60.52, 54.77, 15.44; HRMS (ESI) m/z calcd for $C_{20}H_{18}F_2N_6O$ $[M+H]^+$: 397.1583, found: 397.1582

The target compounds **8b-u** were synthesized by the same procedure as the compound **8a**.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ejmech.2017.10.081.

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