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Discovery of Highly Potent Triazole Antifungal Agents with Piperdine-oxadiazole Side Chains

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Abstract

Due to increasing incidence and mortality of invasive fungal infections, discovery and development of new generations of antifungal agents represents a challenging task. On the basis of our previously reported triazole-benzyloxypiperidinyl lead compound, a series of novel triazole antifungal agents containing piperdine-oxadiazoleside chains were rationally designed and synthesized. Most of the target compounds showed excellent inhibitory activity against clinically important fungal pathogens. In particular, compounds **6g** (MIC = 0.031 μg/mL) and **11b** (MIC = 0.016 μg/mL) were highly active against *Candida albicans* including fluconazole-resistant strains. Moreover, they showed inhibitory activity against hyphal formation with low toxicity, which were promising leads for further development.

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Introduction

There has been a substantial increase in the incidence and mortality of invasive fungal infections (IFIs) over the past two decades.[1] Most patients infected with IFIs are immunocompromised, such as patients with human immunodeficiency virus (HIV) infection and recipients of the organ transplantation, stem cell transplantation, or aggressive antitumor chemotherapy. *Candida albicans* (mortality rate: 20%–40%), *Cryptococcus neoformans* (mortality rate: 50%–90%) and *Aspergillus fumigatus* (mortality rate: 50%–90%) are the three most common species of life-threatening IFIs.[2, 3] Because the immune system of the immunocompromised host cannot effectively clear off the infection, the success of treatment of IFIs is largely dependent on the efficacy of the antifungal agent. Unfortunately, effective antifungal agents are rather limited, particularly in comparison to the number of clinically available antibacterial agents.

Triazole antifungal agents, including fluconazole, voriconazole, itraconazole and posaconazole (**Fig. 1**), are widely used as the first-line antifungal drugs for treatment and prophylaxis of IFIs. Triazole antifungals are inhibitors of lanosterol 14α-demethylase (CYP51), which is a key enzyme in the biosynthesis of ergosterol from lanosterol in the fungal cell membrane.[4] Despite broad use of the triazoles in clinic, they only have achieved modest success in reducing the high mortality rates associatedwith IFIs. Limitations of triazole antifungal agents mainly include severe resistance, narrow antifungal spectrum and therapeutic window, non-optimal pharmacological and pharmacokinetic profiles, drug-related toxicity and undesirable

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drug-drug interactions.[5] Thus, discovery and development of new generations of antifungal agents is an active research area. A number of new triazoles with improved profiles have been reported.[6-10] Among them, two candidate drugs, namely isavuconazole and albaconazole, are under late stages of clinical trials.[11]

Fungal CYP51s from fungal pathogens are transmembrane proteins, whose crystal structures have not been solved up to date. Previously, we constructed three-dimensional models of fungal CYP51s by homology modeling[12-14] and investigated the binding modes of substrate and triazole antifungal agents [12, 15, 16]. Moreover, structural information of CYP51s facilitated the identification of a number of new antifungal triazoles.[17-19] Among them, the triazole-benzyloxypiperidinyl derivative **(Fig. 2)** showed good antifungal activity with a broad spectrum,[20] providing a good lead compound for further optimization. Herein, a series of novel triazole derivatives with piperdine-oxadiazoleside chains were rationally designed and synthesized. Most of the target compounds showed excellent antifungal activity against a variety of fungal pathogens.

Chemistry

As shown in **Scheme 1**, treatment of 1-*tert*-butyl 4-methyl piperidine-1,4-dicarboxylate (**1**) with hydrazine hydrate afforded **2** by a hydrazinolysis reaction. Then, it was reacted with various acylchlorides to give intermediates **3a-o**. Ring closure reaction of intermediates **3a-o** in presence of 4-methylbenzenesulfonic acid (TsCl) and triethylamine (Et_3N) in THF yielded 1,3,4-oxadiazole intermediates **4a-o**. After deprotection of **4a-o**, 1,3,4-oxadiazoles

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5a-o were further reacted with the oxirane intermediate prepared by our previous protocol[16] to afford the target compounds **6a-o** via ring-open reactions. Chemical synthesis of 1,2,4-oxadiazole target compounds **11a-j** is outlined in **Scheme 2**. The construction of 1,2,4-oxadiazole ring was different from that of 1,3,4-oxadiazole. Reaction of hydroxylamine with various nitriles afforded **8a-j**, which was further condensed with 1-(*tert*-Butoxycarbonyl)isonipecoticacidin in the presence of 1-ethyl-3-(3-dimethyllaminopropyl)carbodiimide hydrochloride (EDCI), *N,N*-diisopropylethylamine (DIPEA) and 1-hydroxybenzotriazole (HOBt) to give 1,2,4-oxadiazoles **9a-j**. Finally, target compounds **11a-j** were obtained by a similar protocol as described in **Scheme 1**.

Results and Discussion

Design Rationale

Currently, structural optimization of triazole antifungal agents was focused on variation of the C3-side chains because triazole, difluorophenyl and tertiary alcohol are regarded as essential pharmacophores.[21] Lead compound in this study had a benzyloxypiperidinyl side chain (**Fig. 2**), which was fitted well with the active site of *C. albicans* CYP51 (CACYP51).[20] However, the lead compound has the unstable benzyl ether substructure. Pharmacokinetic study revealed that its *in vivo* half time ($T_{1/2}$) was only about 1 h. Thus, it is highly desirable to design more stable triazoles retaining good antifungal activity. Our previous study revealed that the benzyl ether substructure of the lead compound could be replaced by the substituted 1,2,4-triazoles.[17] Inspired by the results, herein substituted 1,2,4-oxadiazoles and

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1,3,4-oxadiazoles were used as the biosteres of the benzyl ether group to afford target compounds **6a-o** and **11a-j** (**Fig. 2**). The selection of oxadiazole was based on the following considerations. First, oxadiazole is a drug-like privileged structure in many therapeutic drugs and natural products.[22] Second, oxadiazole can be used as a flat, aromatic linker to place substituents in the appropriate orientation[23] and thus favors the interaction of C3-side chain with CYP51. Third, introduction of 1,3,4-oxadiazole ring was proven to be an effective strategy to modulate lipophilicity and improve the characteristics of the *in vivo* metabolic profile.[22]

***In Vitro* Antifungal Activity**

The target compounds were assayed for inhibitory activity against clinically important pathogenic fungi. Fluconazole was used as the reference drug. As shown in **Table 1**, all of the title compounds were highly active against the tested fungal pathogens. There are seven compounds (**6b-d**, **6g-h**, **11a-b**) proven to be more potent than fluconazole against most of the tested fungal strains. Particularly, compounds **6g** (MIC = 0.031 μg/mL) and **11b** (MIC = 0.016 μg/mL) revealed excellent activity against *C. albicans*, which were 8 to 16 fold more active than fluconazole. In contrast, the target compounds generally showed decreased activity against *C. parapsilosis* as well as *C. glabrata*. Cryptococcosis, particularly Cryptococcal meningitisis, is one of the most important fungal infections of humans with high mortality.[24] To our delight, compounds **6c**, **6d**, **6h**, **6n**, **11a** showed better activity (MIC ≤ 0.125 μg/mL) than fluconazole (MIC = 0.25 μg/mL). Invasive aspergillosis is another devastating illness with mortality rates in some patient groups reaching as high as 90%.[25]However,

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fluconazole is inactive against *A. fumigatus* (MIC > 64 μg/mL). Interestingly, most compounds exhibited moderate to good activity against *A. fumigatus*. In particular, compounds **11a** and **11b** revealed the best results (MIC = 2 μg/mL). Furthermore, the target compounds also showed good activity against dematophytes (*T. rubrum* and *M. gypseum*) with MIC values ranging from 0.5 μg/mL to 64 μg/mL, which was significantly more potent than fluconazole. Among the synthesized compounds, **6c**, **6g**, **6h**, **11a** and **11b** showed excellent antifungal activity with a broad spectrum, which were promising leads for the development of new generations of triazole antifungal agents.

Notably, compounds **6g** and **11b** also had potent activity against fluconazole-resistant strains (**Table 2**). Fluconazole-resistant *C. albicans* strains 103, 805 and 0710922 were used in this study. Compounds **6g** and **11b** exhibited antifungal effect, with MIC_{50} values ranging from 0.0625 to 0.25 μg/mL and MIC_{80} values ranging from 4 to 16 μg/mL. In contrast, the MIC_{50} values of fluconazole ranged from 256 to 512 μg/mL, and the MIC_{80} of fluconazole is more than 1024 μg/mL. The results indicated that they might have therapeutic potential to overcome triazole resistance.

Structure-activity Relationships

From the antifungal activity data, preliminary structure-activity relationships (SARs) for both series were obtained. Generally, both of the substituted 1,2,4-oxadiazoles and 1,3,4-oxadiazoles showed broad–spectrum antifungal activity, validating the effectiveness of incorporating oxadiazoles in the side chain of triazole antifungal

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agents. For the substitutions on oxadiazole, there was obvious difference between 1,2,4-oxadiazole and 1,3,4-oxadiazole series. For the 1,2,4-oxadiazole derivatives, the replacement of the phenyl group of compound **6b** with pridinyl group (**6a** and **6l**) led to decrease of the antifungal activity. In contrast, the corresponding furan derivative (**6n)** showed comparable or superior activity to compound **6b**. Substitutions on the phenyl group of **6b** had various effects on the antifungal activity. Introduction of 4-methoxyl (**6f**), 4-trifluoromethyl (**6g**), and 4-fluoro (**6h**) substitutions were favorable for the antifungal activity. Particularly, the 4-trifluoromethyl derivative **6g** showed the best activity against *C. albicans* (MIC = 0.031 μg/mL). Interestingly, the other two fluorine-containing compounds (**6c** and **6d**) also showed excellent antifungal activity. In contrast, introduction of 4-chloro (**6j**), 4-ethyl (**6k**) and 4-nitro (**6o**) substitutions resulted in decreased antifungal activity. Similarly, incorporating 2-methyl (**6e**) and 3-methyl (**6i**) groups or replacing the phenyl group with more steric naphthyl (**6m**) group also had negative effects on the antifungal activity. For the 1,3,4-oxadiazole derivatives, compounds with heterocyclic substitutions were generally more active than the substituted phenyl derivatives with the exception of compound **11b**. The 4-methoxylphenyl derivative **11b** was the most active *C. albicans* inhibitor (MIC = 0.016 μg/mL) among the synthesized compounds. Unlike the 1,2,4-oxadiazole derivatives, the fluorine-containing derivatives (**11f**, **11i** and **11j**) only showed moderate antifungal activity. Among the heterocyclic analogues, the pyridinyl derivative **11a** was the most active one with a broad spectrum.

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Binding Mode of Compounds 6g and 11b with the Active Site of CACYP51

In order to investigate the binding mode of the designed triazoles, two highly active compounds, **6g** and **11b**, were docked into the active site of CACYP51. As shown in **Fig. 3**, the triazole ring and ifluorophenyl group of compounds **6g** and **11b** formed coordination bond and hydrophobic interactions with the heme group and the hydrophobic pocket (Phe126, Ile131 and Tyr132), respectively. The side chains of the two compounds were extended into the S4 pocket of the active site of CACYP51. The oxadiazoyl and piperdinyl groups interacted with Leu376, Met508, Leu121, Ile379 and Tyr118 through hydrophobic and *Van der Waals* interactions. The substitutions on the oxadiazole ring were located into a hydrophobic pocket lined with Tyr505, Gly65 and Tyr64. The magnitude of hydrophobic interactions of various substitutions with this pocket played an important role for the binding affinity and antifungal activity.

Anti-hyphal Activity and Toxicity Assay

We further investigated the activity of **6g** and **11b** against yeast-to-hypha transition of *C. albicans* with fluconazole as the control drug. At 1 μg/mL or higher concentrations, compounds **6g** and **11b** exhibited mild activity against hyphal formation, with less hyphae and more yeasts or pseudohyphal cells compared with the group without drug treatment (**Fig. 4**). No obvious difference was observed between **6g**, **11b** and fluconazole.

Caenorhabditis elegans were used to evaluate the toxicity of compounds **6g** and **11b** at the concentrations ranging from 1 μg/mL to 160 μg/mL. The results revealed that no toxicity was observed at any concentration tested (**Fig. 5**). More specifically,

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no more worm was found dead after drug treatment for 6 days compared with the drug free control group.

Conclusion

In summary, a series of novel triazole antifungal agents were rationally designed and synthesized by incorporating oxadiazole in the C3-side chain. Most of the target compounds showed excellent antifungal activity with a broad spectrum. In particular, compounds **6g** and **11b** were highly active against a variety of fungal pathogens, which can be used as good lead compounds for further development. Molecular docking studies revealed that the oxadiazole derivatives interacted with CACYP51 mainly through hydrophobic and *Van der Waals* interactions. Further evaluation and optimization of the highly potent compounds are in progress.

Experimental Section

General procedure for the synthesis of compounds

All of the ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded on aBruker 300 or 600 spectrometer with TMS as the internal standard. Chemical shifts (δvalues) and coupling constants (*J* values) are given in ppm and Hz, respectively. ESI mass spectra were performed on a Trap LC-MS spectrometer. High-resolution mass spectrometrydata were collected on anAgilent Technologies Q-TOFLC-MS spectrometer.TLC analysis was carried out onsilica gel plates GF254 (Qingdao Haiyang Chemical, China). Silica gelcolumn chromatography was performed with Silica gel 60 G(Qingdao Haiyang Chemical, China). Commercial solvents were used without any pretreatment. The target products were analyzed by HPLC (C18 column,

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460*250 mm, MeOH : H_2O = 70: 30, 254 nm, 0.8 mL/min) and their purity was larger than 96%.

***tert*-Butyl 4-(2-nicotinoylhydrazinecarbonyl)piperidine-1-carboxylate (3a)**

1-*tert*-Butyl 4-methyl piperidine-1,4-dicarboxylate (2.000 g, 8.23 mmol, 1 eq) was dissolved in the mixed solution of hydrazine hydrate and ethanol (1:1, 30 mL) and then stirred overnight at room temperature. After evaporating the ethanol, the residue was poured into water, extracted with DCM (3 × 50 mL), dried with Na_2SO_4, filtered and evaporated under reduced pressure to give **2** as white solid (1.80 g, 95%). Then, product **2** (1.800 g, 7.40 mmol, 1 eq) and Et_3N (1.120 g, 11.10 mmol, 1.5 eq) were dissolved in DCM (180 mL), and was slowly added nicotinoyl chloride (1.980 g, 11.10 mmol, 1.5 eq) under ice bath. The reaction solution was stirred at room temperature for 2 h. After evaporation, the crude product was purified by silica gel column chromatography (eluents: EtOAc : MeOH = 50:1-30:1) to afford compound **3a** as white solid (1.80 g, 70%). ^{1}HNMR (600 MHz, d_6-DMSO) δ 10.53 (s, 1H, NHNH), 9.88 (s, 1H, NHNH), 9.01 (d, J = 2.2 Hz, 1H, pyridine-H), 8.75 (dd, J = 1.6 Hz, 4.8 Hz, 1H, pyridine-H), 8.20 (dt, J = 1.7 Hz, 7.9 Hz, 1H, pyridine-H), 7.54 (dd, J = 4.8 Hz, 7.9 Hz, 1H, pyridine-H), 3.96 (m, 2H, piperidin-2-CH_2), 2.79 (m, 2H, piperidin- 6-CH_2), 2.47 (m, 1H, piperidin-4-CH), 1.73 (m, 2H, piperidin-5-CH_2), 1.48 (m, 2H, piperidin-3-CH_2), 1.40 (s, 9H, $C(CH_3)_3$); MS (ESI) m/z: 347.3 (M-H).

***tert*-Butyl 4-(5-(pyridin-3-yl)-1,3,4-oxadiazol-2-yl)piperidine-1-carboxylate (4a)**

Compound **3a** (1.800 g, 5.17 mmol, 1 eq) and Et_3N (3.140 g, 31.02 mmol, 6 eq) were dissolved in dry THF (100 mL), and were added *p*-toluene sulfonyl chloride (2.960 g,

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15.51 mmol, 3 eq). The mixture was stirred overnight at 50 ℃. The crude product was purified by silica gel column chromatography (eluents: EtOAc : MeOH = 80:1-50:1) to give **4a** as faint yellow solid (1.200 g, 71%)**.** ^{1}HNMR (600 MHz, d_6-DMSO) δ 9.15 (s, 1H, pyridine-H), 8.79 (dd, J = 4.8 Hz, 1H, pyridine-H), 8.35 (d, J = 8.0 Hz, 1H, pyridine-H), 7.62 (dd, J = 4.8 Hz, 8.0 Hz, 1H, pyridine-H), 3.93 (m, 2H, piperidin-2-CH_2), 3.29 (m, 1H, piperidin-4-CH), 3.00 (m, 2H, piperidin-6-CH_2), 2.07 (m, 2H, piperidin-3-CH_2), 1.68 (m, 2H, piperidin-5-CH_2), 1.41 (s, 9H, $C(CH_3)_3$); HRMS (ESI) calcd for $C_{17}H_{22}N_4O_3$ $[M+H]^+$ = 331.1765, found 331.1765.

2-(2,4-Ddifluorophenyl)-1-(4-(5-(pyridin-3-yl)-1,3,4-oxadiazol-2-yl)piperidin-1-yl)-3-(1*H*-1,2,4-triazol-1-yl)propan-2-ol (6a)

A solution of intermediate **4a** (0.240 g, 0.72 mmol, 1 eq) and trifluoroacetic acid (3.0 mL) in DCM (50 mL) were stirred at room temperature for 10 h. Then, the reaction mixture was neutralized by adding aqueous K_2CO_3 (3.000 g) slowly. The DCM layer was separated, and the water layer was extracted with DCM. The organic layer was combined, dried over anhydrous Na_2SO_4 and filtrated. Compound **5a** was obtained by evaporating under reduced pressure (yellow solid, 0.15 g, 91%). The product was used directly in the next step without further purification. A solution of **5a** (0.140 g, 0.61 mmol, 1 eq), oxirane intermediate（0.20 g, 1 eq）and Et_3N (1 mL) in EtOH were refluxed at 78℃ for 5 h. The solvent was removed under the reduced pressure and the residue was purified by silica gel column chromatography (eluents: DCM : MeOH = 30:1) to afford target product **6a** (yellow powder, 0.15 g, 52.8%). ^{1}HNMR (300 MHz, $CDCl_3$) δ 9.22 (d, J = 1.5 Hz, 1H, pyridine-H), 8.76 (dd, J = 1.5, 4.8 Hz, 1H,

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pyridine-H), 8.32(dt, *J* = 1.7, 7.9 Hz, 1H, pyridine-H),8.16 (s, 1H, TriazC_3-H), 7.80 (s, 1H, TriazC_5-H), 7.51 - 7.64 (m, 1H, pyridine-H), 7.45 (dd, *J* = 4.8, 8.2 Hz, 1H, Ar-H), 6.83 (m, 2H, Ar-H), 4.56 (d, *J* = 14.6 Hz, 1H, C_1-Ha), 4.50 (d, *J* = 14.6 Hz, 1H, C_1-Hb), 3.12 (d, *J* = 13.8 Hz, 1H, C_3-Ha), 2.89 – 3.00 (m, 1H, C_3-Hb), 2.69 (d, *J* = 13.8 Hz, 2H, piperidin-CH_2), 2.38 -2.58 (m, 2H, piperidin-CH_2), 2.23 (t, *J* = 11.3 Hz, 1H, piperidin-4-CH), 2.07 (t, *J* = 11.6 Hz, 1H, piperidin-CH_2), 1.72-2.00 (m, 3H, piperidin-CH_2); ^{13}CNMR (75 MHz, $CDCl_3$): δ 169.0, 162.7 ($^1J_{CF}$ = 248.2 Hz), 162.6, 158.9 ($^1J_{CF}$ = 247.1 Hz), 152.4, 151.1, 147.7, 144.7, 134.1, 129.3, 126.1, 123.8, 120.4, 111.6, 104.3, 72.1, 62.5, 56.1, 54.5, 53.4, 32.7, 29.7, 29.2; MS (ESI) m/z: 468.38 (M+H). HRMS (ESI) calcd for $C_{23}H_{23}F_2N_7O_2$ $[M+H]^+$ = 468.1962, found 468.1960. The synthetic procedure for compounds **6b-o** was similar to the synthesis of compound **6a**.

2-(2,4-Difluorophenyl)-1-(4-(5-phenyl-1,3,4-oxadiazol-2-yl)piperidin-1-yl)-3-(1*H*-1,2,4-triazol-1-yl)propan-2-ol (6b)

^{1}HNMR (300 MHz, $CDCl_3$) δ 8.16 (s, 1H, TriazC_3-H), 8.00 (dd, *J* = 2.1, 7.8 Hz, 2H, Ar-H), 7.79 (s, 1H, TriazC_5-H), 7.39-7.65 (m, 4H, Ar-H), 6.82 (m, 2H, Ar-H), 4.56 (d, *J* = 14.6 Hz, 1H, C_1-Ha), 4.50 (d, *J* = 14.6 Hz, 1H, C_1-Hb), 3.11 (d, *J* = 13.6 Hz, 1H, C_3-Ha), 2.91 (d, *J* = 4.5 Hz, 1H, C_3-Hb), 2.69 (d, *J* = 12.2 Hz, 2H, piperidin-CH_2), 2.47 (m, 2H, piperidin-CH_2), 2.22 (t, *J* = 10.1 Hz, 1H, piperidin-4-CH), 1.78-2.11 (m, 4H, piperidin-CH_2); ^{13}CNMR (75 MHz, $CDCl_3$): δ 168.4, 164.6, 162.8 ($^1J_{CF}$ = 246.0 Hz), 158.9 ($^1J_{CF}$ = 246.0 Hz), 151.0, 144.6, 131.6, 129.3, 129.0, 126.7, 126.1, 123.8, 111.6, 104.3, 72.1, 62.5, 56.3, 54.5, 53.4, 32.6, 29.6, 29.2; MS (ESI) m/z: 489.3

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(M+Na). HRMS (ESI) calcd for $C_{24}H_{24}F_2N_6O_2$ $[M+H]^+$ = 467.1933, found 467.1932.

2-(2,4-Difluorophenyl)-1-(4-(5-(2,6-difluorophenyl)-1,3,4-oxadiazol-2-yl)piperidin -1-yl)-3-(1*H*-1,2,4-triazol-1-yl)propan-2-ol (6c)

^{1}HNMR (300 MHz, $CDCl_3$) δ 8.15 (s, 1H, TriazC_3-H), 7.79 (s, 1H, TriazC_5-H), 7.72 (m, 1H, Ar-H), 7.56 (m, 1H, Ar-H), 7.21 (t, *J* = 5.9 Hz, 2H, Ar-H), 6.82 (m, 2H, Ar-H), 5.20 (brs, 1H, OH), 4.56 (d, *J* = 14.6 Hz, 1H, C_1-Ha), 4.50 (d, *J* = 14.6 Hz, 1H, C_1-Hb), 3.11 (d, *J* = 13.2 Hz, 1H, C_3-Ha), 2.95 (m, 1H, C_3-Hb), 2.69 (d, *J* = 13.2 Hz, 2H, piperidin-CH_2), 2.47 (t, *J* = 9.8 Hz, 2H, piperidin-CH_2), 2.21 (t, *J* = 9.8 Hz, 1H, piperidin-4-CH), 1.76 - 2.11 (m, 4H, piperidin-CH_2); ^{13}CNMR (75 MHz, $CDCl_3$): δ 169.1, 162.4 ($^1J_{CF}$ = 249.6 Hz), 160.6, 158.9 ($^1J_{CF}$ = 247.6 Hz), 158.3 ($^1J_{CF}$ = 244.7 Hz), 155.9 ($^1J_{CF}$ = 254.5 Hz), 151.0, 144.6, 129.3, 126.1, 120.0, 118.4, 115.9, 113.4, 111.6, 104.3, 72.1, 62.5, 56.2, 54.5, 53.4, 32.5, 29.6, 29.1; MS (ESI) m/z: 525.3 (M+Na). HRMS (ESI) calcd for $C_{24}H_{22}F_4N_6O_2$ $[M+H]^+$ = 503.1724, found 503.1724.

2-(2,4-Difluorophenyl)-1-(4-(5-(2-fluorophenyl)-1,3,4-oxadiazol-2-yl)piperidin-1-yl)-3-(1*H*-1,2,4-triazol-1-yl)propan-2-ol (6d)

^{1}HNMR (300 MHz, $CDCl_3$) δ 8.15 (s,1H, TriazC_3-H), 8.01 (t, *J* = 7.3 Hz, 1H, Ar-H), 7.78(s,1H, TriazC_5-H), 7.53 (m, 2H, Ar-H), 7.25 (m, 2H, Ar-H), 6.80 (m, 2H, Ar-H), 4.56 (d, *J* = 14.6 Hz, 1H, C_1-Ha), 4.50 (d, *J* = 14.6 Hz, 1H, C_1-Hb), 3.10 (d, *J* = 13.3 Hz, 1H, C_3-Ha), 2.93 (m, 1H, C_3-Hb), 2.68 (d, *J* = 13.8 Hz, 2H, piperidin-CH_2), 2.47 (m, 2H, piperidin-CH_2), 2.22 (t, *J* = 11.6 Hz, 1H, piperidin-4-CH), 2.07 (d, *J* = 11.6 Hz, 1H, piperidin-CH_2), 1.76 - 2.11 (m, 3H, piperidin-CH_2); ^{13}CNMR (75 MHz, $CDCl_3$): δ 168.8, 162.8 ($^1J_{CF}$ = 248.5 Hz), 161.5, 159.8 ($^1J_{CF}$ = 251.0 Hz), 158.9 ($^1J_{CF}$

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= 244.8 Hz), 151.0, 144.6, 133.4, 129.7, 129.3, 126.0, 124.6, 116.9 ($^2J_{CF}$ = 20.2 Hz), 112.3, 111.5, 104.3, 72.0, 62.5, 56.2, 54.5, 53.4, 32.5, 29.5, 29.1; MS (ESI) m/z: 507.3 (M+Na). HRMS (ESI) calcd for $C_{24}H_{23}F_3N_6O_2$ $[M+H]^+$ = 485.1825, found 485.1824.

2-(2,4-Difluorophenyl)-1-(4-(5-(o-tolyl)-1,3,4-oxadiazol-2-yl)piperidin-1-yl)-3-(1*H*-1,2,4-triazol-1-yl)propan-2-ol (6e)

^{1}HNMR (300 MHz, d_6-DMSO) δ 8.18 (s, 1H, TriazC$_3$-H), 7.86 (d, *J* = 7.7 Hz, 1H, Ar-H), 7.79 (s, 1H, TriazC$_5$-H), 7.66 (m, 1H, Ar-H), 7.27 - 7.48 (m, 3H, Ar-H), 6.85 (m, 2H, Ar-H), 5.66(brs, 1H, OH), 4.52 (m, 2H, C$_1$-H), 2.86 - 3.32 (m, 4H, C$_3$-H, piperidin-CH$_2$), 2.68 (s, 1H, piperidin-4-CH), 2.37 - 2.61 (m, 2H, piperidin-CH$_2$), 2.14 - 2.37 (m, 2H, piperidin-CH$_2$), 1.88 - 2.13 (m, 3H, CH$_3$) ; MS (ESI) m/z: 481.4 (M+1). HRMS (ESI) calcd for $C_{25}H_{26}F_2N_6O_2$ $[M+H]^+$ = 481.2142, found 481.2142.

2-(2,4-Difluorophenyl)-1-(4-(5-(4-methoxyphenyl)-1,3,4-oxadiazol-2-yl)piperidin-1-yl)-3-(1*H*-1,2,4-triazol-1-yl)propan-2-ol (6f)

^{1}HNMR (300 MHz, d_6-DMSO) δ 8.31 (s,1H, TriazC$_3$-H), 7.90 (d, *J* = 8.8 Hz, 2H, Ar-H), 7.75 (s, 1H, TriazC$_5$-H), 7.41 (m, 1H, Ar-H), 7.20 (m, 1H, Ar-H), 7.12 (d, *J* = 8.8 Hz, 2H, Ar-H), 6.97 (t, *J* = 8.5 Hz, 1H, Ar-H), 5.66 (brs, 1H, OH), 4.58 (m, 2H, C$_1$-H), 3.84 (s, 3H, OCH$_3$), 2.88 (d, *J* = 15.3 Hz, 2H, C$_3$-H), 2.80 (d, *J* = 10.9 Hz, 1H, piperidin-H), 2.71 (d, *J* = 13.8 Hz, 2H, piperidin-H), 2.29 (t, *J* = 8.7 Hz, 2H, piperidin-H), 1.91 (m, 2H, piperidin-H), 1.68 (q, *J* = 10.9, 2H, piperidin-H);^{13}CNMR (75 MHz, CDCl$_3$): δ 167.9, 164.5, 162.7 ($^1J_{CF}$ = 249.8 Hz), 162.2, 158.8 ($^1J_{CF}$ = 244.3 Hz), 151.0, 144.9, 129.3, 128.5, 126.1, 116.4, 114.4, 111.5, 104.4, 72.1, 62.6, 56.3, 55.4, 54.6, 53.4, 32.7, 29.6, 29.2; MS (ESI) m/z: 497.3 (M+1). HRMS (ESI) calcd for

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$C_{25}H_{26}F_2N_6O_3$ $[M+H]^+$ = 497.2022, found 497.2022.

2-(2,4-Difluorophenyl)-1-(1*H*-1,2,4-triazol-1-yl)-3-(4-(5-(4-(trifluoromethyl)phenyl)-1,3,4-oxadiazol-2-yl)piperidin-1-yl)propan-2-ol (6g)

^{1}HNMR (300 MHz, $CDCl_3$) δ 8.14 (s, 2H, Ar-H), 8.11 (s, 1H, TriazC_3-H), 7.78 (s, 1H, TriazC_5-H), 7.74 (d, *J* = 8.3 Hz, 2H, Ar-H), 7.56 (m, 1H, Ar-H), 6.81 (m, 2H, Ar-H), 5.18 (brs, 1H, OH), 4.53 (m, 2H, C_1-H), 3.11 (d, *J* = 13.6 Hz, 1H, C_3-Ha), 2.94 (m, 1H, C_3-Hb), 2.70 (d, *J* = 13.6 Hz, 2H, piperidin-CH_2), 2.48 (m, 2H, piperidin-CH_2), 2.23 (t, *J* = 10.8 Hz, 1H, piperidin-4-CH), 2.08 (m, 1H, piperidin-CH_2), 1.76 - 1.99 (m, 3H, piperidin-CH_2); ^{13}CNMR (75 MHz, $CDCl_3$): δ 169.0, 163.5, 162.6 ($^1J_{CF}$ = 249.5 Hz), 158.7 ($^1J_{CF}$ = 248.3 Hz), 151.0, 144.7, 133.3, 129.3, 127.1, 126.1, 125.3, 121.7, 111.6, 104.2, 71.9, 62.2, 56.1, 54.4, 53.3, 32.7, 29.6, 29.2; MS (ESI) m/z: 535.3 (M+H). HRMS (ESI) calcd for $C_{25}H_{23}F_5N_6O_2$ $[M+H]^+$ = 535.1821, found 535.1821.

2-(2,4-Difluorophenyl)-1-(4-(5-(4-fluorophenyl)-1,3,4-oxadiazol-2-yl)piperidin-1-yl)-3-(1*H*-1,2,4-triazol-1-yl)propan-2-ol (6h)

^{1}HNMR (300 MHz, $CDCl_3$) δ 8.14 (s, 1H, TriazC_3-H), 7.94-8.05 (m, 2H, Ar-H), 7.78 (s, 1H, TriazC_5-H), 7.55 (m, 1H, Ar-H), 7.16 (t, *J* = 8.4 Hz, 2H, Ar-H), 6.73 - 6.87 (m, 2H, Ar-H), 4.52 (m, 2H, C_1-H), 3.09 (d, *J* = 13.2 Hz, 1H, C_3-Ha), 2.90 (m, 1H, C_3-Hb), 2.68 (d, *J* = 13.4 Hz, 2H, piperidin-CH_2), 2.46 (m, 2H, piperidin-CH_2), 2.20 (t, *J* = 11.2 Hz, 1H, piperidin-4-CH), 2.05 (m, 1H, piperidin-CH_2), 1.74 - 1.94 (m, 3H, piperidin-CH_2); ^{13}CNMR (75 MHz, $CDCl_3$): δ 168.4, 164.7 ($^1J_{CF}$ = 252.4 Hz), 163.8, 162.7 ($^1J_{CF}$ = 250.2 Hz), 159.0 ($^1J_{CF}$ = 247.3 Hz), 151.0, 144.6, 129.3, 129.1, 128.9, 126.1, 120.2, 116.5, 116.2, 111.6, 104.3, 72.1 , 62.4, 56.2, 54.5, 53.4, 32.6, 29.6, 29.2;

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MS (ESI) m/z: 485.3 (M+H). HRMS (ESI) calcd for $C_{24}H_{23}F_3N_6O_2$ $[M+H]^+$ = 485.1825, found 485.1825.

2-(2,4-Difluorophenyl)-1-(4-(5-(m-tolyl)-1,3,4-oxadiazol-2-yl)piperidin-1-yl)-3-(1*H*-1,2,4-triazol-1-yl)propan-2-ol (6i)

^{1}HNMR (300 MHz, $CDCl_3$) δ 8.14 (s, 1H, TriazC$_3$-H), 7.81(s, 1H, TriazC$_5$-H), 7.78 (s, 2H, Ar-H), 7.55 (q, *J* = 9.0 Hz, 1H, Ar-H), 7.33 (m, 2H, Ar-H), 6.80 (m, 2H, Ar-H), 4.52 (m, 2H, C_1-H), 3.10 (d, *J* = 14.0 Hz, 1H, C_3-Ha), 2.90 (m, 1H, C_3-Hb), 2.69 (d, *J* = 12.7 Hz, 2H, piperidin-CH_2), 2.46 (m, 2H, piperidin-CH_2), 2.40 (s, 3H, CH_3), 2.21 (t, *J* = 11.8 Hz, 1H, piperidin-4-CH), 2.06 (m, 1H, piperidin-CH_2), 1.77 - 1.99 (m, 3H, piperidin-CH_2) ; ^{13}CNMR (75 MHz, $CDCl_3$): δ 168.2, 164.8, 162.6 ($^1J_{CF}$ = 249.1 Hz), 158.8 ($^1J_{CF}$ = 246.8 Hz), 151.0, 144.7, 138.9, 132.4, 129.3, 128.9, 127.3, 125.9, 123.9, 123.7, 111.6, 104.3, 72.0, 62.4, 56.3, 54.5, 53.4, 32.4, 29.5, 29.1, 21.3; MS (ESI) m/z: 481.4 (M+H). HRMS (ESI) calcd for $C_{25}H_{26}F_2N_6O_2$ $[M+H]^+$ = 481.2131, found 481.2131.

1-(4-(5-(4-Chlorophenyl)-1,3,4-oxadiazol-2-yl)piperidin-1-yl)-2-(2,4-difluorophenyl)-3-(1*H*-1,2,4-triazol-1-yl)propan-2-ol (6j)

^{1}HNMR (300 MHz, $CDCl_3$) δ 8.18 (s, 1H, TriazC$_3$-H), 7.94 (d, *J* = 8.8 Hz, 2H, Ar-H), 7.80 (s, 1H, TriazC$_5$-H), 7.56 (q, *J* = 9.4 Hz, 1H, Ar-H), 7.46 (d, *J* = 8.5 Hz, 2H, Ar-H), 6.81 (m, 2H, Ar-H), 4.53 (m, 2H, C_1-H), 3.12 (d, *J* = 13.6 Hz, 1H, C_3-Ha), 2.92 (m, 1H, C_3-Hb), 2.71 (d, *J* = 12.8 Hz, 2H, piperidin-CH_2), 2.48 (m, 2H, piperidin-CH_2), 2.23 (m, 1H, piperidin-4-CH), 1.82-2.08 (m, 4H, piperidin-CH_2); ^{13}CNMR (75 MHz, $CDCl_3$): δ 168.5, 164.3, 162.6 ($^1J_{CF}$ = 248.7 Hz), 159.0 ($^1J_{CF}$ = 248.7 Hz), 150.9,

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144.7, 137.9, 129.4, 129.3, 128.0, 126.0, 122.3, 111.6, 104.3, 72.0, 62.4, 56.3, 54.5, 53.4, 32.6, 29.6, 29.2; MS (ESI) m/z: 501.4 (M+H). HRMS (ESI) calcd for $C_{24}H_{23}ClF_2N_6O_2$ $[M+H]^+$ = 501.1523, found 501.1523.

2-(2,4-Difluorophenyl)-1-(4-(5-(4-ethylphenyl)-1,3,4-oxadiazol-2-yl)piperidin-1-yl)-3-(1*H*-1,2,4-triazol-1-yl)propan-2-ol (6k)

^{1}HNMR (300 MHz, $CDCl_3$) δ 8.44 (s, 1H, TriazC$_3$-H), 7.91 (d, *J* = 8.1 Hz, 1H, Ar-H), 7.70 (s, 1H, TriazC$_5$-H), 7.53 (d, *J* = 7.2 Hz, 1H, Ar-H), 7.31 (d, *J* = 8.0 Hz, 2H, Ar-H), 7.04 (s, 1H, Ar-H), 6.82 (t, *J* = 8.8 Hz, 2H, Ar-H), 4.57 (m, 2H, C$_1$-H), 3.17 (m, 1H, C$_3$-Ha), 2.98 (m, 1H, C$_3$-Hb), 2.72 (d, *J* = 7.4 Hz, 2H, piperidin-CH_2), 2.43 (m, 2H, piperidin-CH_2), 2.33 (m, 1H, piperidin-4-CH), 2.22 (m, *J* = 7.4 Hz, 2H, C$\underline{H}_2$CH$_3$), 1.91 - 2.06 (m, 4H, piperidin-CH_2), 1.17 (m, 3H, $CH_2C\underline{H}_3$); MS (ESI) m/z: 495.5 (M+H). HRMS (ESI) calcd for $C_{24}H_{23}F_3N_6O_2$ $[M+H]^+$ = 495.2217, found 495.2217.

2-(2,4-Difluorophenyl)-1-(4-(5-(pyridin-4-yl)-1,3,4-oxadiazol-2-yl)piperidin-1-yl)-3-(1*H*-1,2,4-triazol-1-yl)propan-2-ol (6l)

^{1}HNMR (300 MHz, $CDCl_3$) δ 8.81 (d, *J* = 6.4 Hz, 2H, pyridine-H), 8.28 (s, 1H, TriazC$_3$-H), 7.88 (d, *J* = 6.0 Hz, 2H, pyridine-H), 7.83 (s, 1H, TriazC$_5$-H), 7.55 (m, 1H, Ar-H), 6.83 (m, 2H, Ar-H), 4.56 (m, 2H, C$_1$-H), 3.15 (d, *J* = 13.6 Hz, 1H, C$_3$-Ha), 2.98 (m, 1H, C$_3$-Hb), 2.73 (m, 2H, piperidin-CH_2), 2.53 (m, 2H, piperidin-CH_2), 2.08 (s, 1H, piperidin-4-CH), 1.92 - 2.04 (m, 4H, piperidin-CH_2); MS (ESI) m/z: 468.4 (M+H). HRMS (ESI) calcd for $C_{23}H_{23}F_2N_7O_2$ $[M+H]^+$ = 468.1936, found 468.1936.

2-(2,4-Difluorophenyl)-1-(4-(5-(naphthalen-1-yl)-1,3,4-oxadiazol-2-yl)piperidin-1-yl)-3-(1*H*-1,2,4-triazol-1-yl)propan-2-ol (6m)

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^{1}HNMR (300 MHz, $CDCl_3$) δ 9.16 (d, *J* = 8.4 Hz, 1H, naphtalinum-H),8.19 (s, 1H, TriazC_3-H), 8.10 (d, *J* = 7.4 Hz, 1H, naphtalinum-H), 8.01 (d, *J* = 8.4 Hz, 1H, naphtalinum-H), 7.92 (d, *J* = 7.9 Hz, 1H, naphtalinum-H), 7.80 (s, 1H, TriazC_5-H), 7.66 (t, *J* = 7.5 Hz, 1H, Ar-H), 7.56 (q, *J* = 7.9 Hz, 3H, naphtalinum-H), 6.81 (m, 2H, Ar-H), 4.53 (m, 2H, C_1-H), 3.13 (d, *J* = 13.6 Hz, 1H, C_3-Ha), 2.99 (m, 1H, C_3-Hb), 2.71 (d, *J* = 13.6 Hz, 2H, piperidin-CH_2), 2.50 (m, 2H, piperidin-CH_2), 2.25 (t, *J* = 11.5 Hz, 1H, piperidin-4-CH), 1.86 - 2.13 (m, 4H, piperidin-CH_2); ^{13}CNMR (75 MHz, $CDCl_3$): δ 168.0, 164.6, 162.7 ($^1J_{CF}$ = 249.4 Hz), 158.7 ($^1J_{CF}$ = 245.9 Hz), 151.0, 144.7, 133.8, 132.5, 130.0, 129.3, 128.6, 128.2, 128.1, 126.7, 126.1, 126.0, 124.7, 120.5, 111.6, 104.3, 72.1, 62.5, 56.3, 54.6, 53.5, 32.6, 29.7, 29.3; MS (ESI) m/z: 517.4 (M+H). HRMS (ESI) calcd for $C_{28}H_{26}F_2N_6O_2$ $[M+H]^+$ = 517.2138, found 517.2138.

2-(2,4-Difluorophenyl)-1-(4-(5-(furan-2-yl)-1,3,4-oxadiazol-2-yl)piperidin-1-yl)-3-(1*H*-1,2,4-triazol-1-yl)propan-2-ol (6n)

^{1}HNMR (300 MHz, $CDCl_3$) δ 8.24 (s, 1H, TriazC_3-H), 7.81 (s, 1H, TriazC_5-H), 7.55 (m, 2H, furan-H), 7.12 (s, 1H, Ar-H), 6.82 (s, 2H, Ar-H), 6.57 (s, 1H, furan-H), 4.53 (m, 2H, C_1-H), 3.13 (d, *J* = 12.7 Hz, 1H, C_3-Ha), 2.92 (m, 1H, C_5-Ha), 2.69 (s, 2H, piperidin-CH_2), 2.49 (s, 2H, piperidin-CH_2), 2.23 (s, 1H, piperidin-4-CH), 1.75 - 2.11 (m, 4H, piperidin-CH_2); ^{13}CNMR (75 MHz, $CDCl_3$): δ 167.6, 162.6 ($^1J_{CF}$ = 249.6 Hz), 158.6 ($^1J_{CF}$ = 249.0 Hz), 157.5, 150.9, 145.6, 144.8, 139.3, 129.3, 125.9, 113.8, 112.1, 111.6, 104.3, 71.9, 62.5, 56.3, 54.4, 53.4, 32.4, 29.6, 29.0; MS (ESI) m/z: 457.4 (M+H). HRMS (ESI) calcd for $C_{22}H_{22}F_2N_6O_3$ $[M+H]^+$ = 457.1725, found 457.1725.

2-(2,4-Difluorophenyl)-1-(4-(5-(4-nitrophenyl)-1,3,4-oxadiazol-2-yl)piperidin-1-yl

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)-3-(1*H*-1,2,4-triazol-1-yl)propan-2-ol (6o)

^{1}HNMR (300 MHz, $CDCl_3$) δ 8.80 (s, 1H, Ar-H), 8.37 (d, *J* = 7.2, 1H, Ar-H), 8.15 (s, 1H, TriazC_3-H), 7.79 (s, 1H, TriazC_5-H), 7.71 (t, *J* = 8.1 Hz, 1H, Ar-H), 7.57 (q, *J* = 8.9 Hz, 1H, Ar-H), 6.81 (m, 2H, Ar-H), 4.53 (m, 2H, C_1-H), 3.13 (d, *J* = 13.4 Hz, 1H, C_3-Ha), 2.92 (m, 1H, C_3-Hb), 2.71 (d, *J* = 12.6 Hz, 2H, piperidin-CH_2), 2.49 (m, 2H, piperidin-CH_2), 2.23 (m, 1H, piperidin-4-CH), 1.83 - 2.10 (m, 4H, piperidin-CH_2); ^{13}CNMR (75 MHz, $CDCl_3$): δ 169.3, 162.8, 162.7 ($^1J_{CF}$ = 249.6 Hz), 158.8 ($^1J_{CF}$ = 245.6 Hz), 151.0, 148.6, 144.7, 132.3, 130.4, 129.3, 126.0, 126.0, 125.5, 121.6, 111.5, 104.3, 72.1, 62.5, 56.2, 54.5, 53.4, 32.7, 29.6, 29.2; MS (ESI) m/z: 512.4 (M+H). HRMS (ESI) calcd for $C_{24}H_{23}F_2N_7O_4$ $[M+H]^+$ = 512.1843, found 512.1843.

***N*′-hydroxynicotinimidamide (8a)**

A mixture of nicotinonitrile (0.67 g, 6.44 mmol, 1 eq) and NH_2OH·HCl (1.11 g, 16.10 mmol, 2.5 eq) in ethanol (20 mL) were refluxed for 8h. After cooling, white solid was precipitated. After filtration, the residue was recrystallized from water-isopropanol (1:9) to give **8a** (0.79 g, 89%). ^{1}HNMR (600 MHz, d_6-DMSO) δ 9.88 (s,1H, OH), 8.88 (d, *J* = 2.3 Hz, 1H, pyridine-H), 8.57 (dd, *J* = 1.6 Hz, 4.8 Hz, 1H, pyridine-H), 8.03 (dt, *J* = 1.7 Hz, 8.0 Hz, 1H, pyridine-H), 7.41 (dd, *J* = 4.8 Hz, 8.0 Hz, 1H, pyridine-H), 6.00 (brs, 2H, NH_2); MS (ESI) m/z: 138.2 (M+H).

***tert*-Butyl 4-(3-(pyridin-3-yl)-1,2,4-oxadiazol-5-yl)piperidine-1-carboxylate (9a)**

1-(*tert*-Butoxycarbonyl)piperidine-4-carboxylic acid (1.33 g, 5.80 mmol, 1 eq), HOBt (0.94 g, 6.96 mmol, 1.2 eq), EDCI (1.67 g, 8.70 mmol, 1.5 eq) and *N*,*N*-diisopropylethylamine (1.50 g, 11.60 mmol, 2 eq) were dissolved in dioxane

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and stirred at room temperature for 15 min. Subsequently, the solution was added **8a** (0.79 g, 5.80 mmol, 1 eq) and the resulting mixture was stirred for about 10 h at 100℃. After evaporating the solvent, the residue was dissolved in ethyl acetate and washed with water (20 mL × 2), aqueous NaOH (20 mL × 2), saturated NaCl (20 mL × 2), dried over Na_2SO_4, and filtrated. The solvent was evaporated under reduced pressure and the crude product was purified by silica gel column chromatography (eluents: DCM : MeOH = 80:1-50:1) to give **9a** as a white solid (0.58 g, yield 31%). ^{1}HNMR (600 MHz, d_6-DMSO) δ 9.16 (d, J = 2.2 Hz, 1H, pyridine-H), 8.78 (d, J = 1.7 Hz, 4.8 Hz, 1H, pyridine-H), 8.35 (dt, J = 1.8 Hz, 8.5 Hz, 1H, pyridine-H), 7.61 (dd, J = 4.8 Hz, 8.0 Hz, 1H, pyridine-H), 3.96 (m, 2H, piperidin-CH_2); 3.39 (m, 1H, piperidin-4-CH), 3.00 (m, 2H, piperidin-CH_2), 2.09 (m, 2H, piperidin-CH_2), 1.68 (m, 2H, piperidin-CH_2), 1.42 (s, 9H, $C(CH_3)_3$); MS (ESI) m/z: 331.4 (M+H).

2-(2,4-Difluorophenyl)-1-(4-(3-(pyridin-3-yl)-1,2,4-oxadiazol-5-yl)piperidin-1-yl)-3-(1*H*-1,2,4-triazol-1-yl)propan-2-ol (11a)

The synthetic protocol of target compounds **11a-j** was similar to that of compound **6a**. **11a** (yellowish pink powder, 0.18 g, 43% for two steps) was obtained from **9a** (0.30 g, 0.89 mmol, 1 eq). ^{1}HNMR (600 MHz, $CDCl_3$) δ 9.28 (d, J = 2.2 Hz, 1H, pyridine-H), 8.73 (dd, J = 1.8 Hz, 5.0 Hz, 1H, pyridine-H), 8.32 (dt, J = 1.8 Hz, 1H, pyridine-H), 8.15 (s, 1H, TriazC_3-H), 7.80 (s, 1H, TriazC_5-H), 7.58 (m, 1H, Ar-H), 7.41 (dd, J = 4.8 Hz, 8.0 Hz, 1H, pyridine-H), 6.83 (m, 2H, Ar-H), 5.20 (brs, 1H, OH), 4.57 (d, J = 14.1 Hz, 1H, C_1-Ha), 4.52 (d, J = 14.1 Hz, 1H, C_1-Hb), 3.11 (d, J = 13.9 Hz, 1H, C_3-Ha), 2.96 (m, 1H, piperidin-CH_2), 2.71 (d, J = 13.9 Hz,1H, C_3-Hb), 2.69 (m, 1H,

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piperidin-CH_2), 2.49 (m, 2H, piperidin-CH_2), 2.25 (t, J = 11.4 Hz, 1H, piperidin-4-CH), 1.80-2.09 (m, 4H, piperidin-CH_2); ^{13}CNMR (75 MHz, $CDCl_3$): δ 181.9, 166.2, 162.7 ($^1J_{CF}$ = 248.8 Hz), 158.9 ($^1J_{CF}$ = 245.3 Hz), 151.9, 151.0, 148.6, 144.6, 134.6, 129.2, 126.0, 123.6, 123.0, 111.7, 104.6, 72.1, 62.4, 56.2, 54.5, 53.4, 33.6, 29.6, 29.2; MS (ESI) m/z: 468.4 (M+H). HRMS (ESI) calcd for $C_{23}H_{23}F_2N_7O_2$ $[M+H]^+$ = 468.1917, found 468.1917. The synthetic procedure for compounds **11b-j** was similar to the synthesis of compound **11a**.

2-(2,4-Difluorophenyl)-1-(4-(3-(4-methoxyphenyl)-1,2,4-oxadiazol-5-yl)piperidin-1-yl)-3-(1*H*-1,2,4-triazol-1-yl)propan-2-ol (11b)

^{1}HNMR (300 MHz, $CDCl_3$) δ 8.21 (s, 1H, TriazC$_3$-H), 7.97 (d, J = 9.1 Hz, 2H, Ar-H), 7.80(s, 1H, TriazC$_5$-H), 7.55 (q, J = 9.4 Hz, 1H, Ar-H), 6.96 (d, J = 8.5 Hz, 2H, Ar-H), 6.82 (m, 2H, Ar-H), 5.20 (brs, 1H, OH), 4.53 (m, 2H, C_1-H), 3.85 (s, 3H, OCH_3), 3.11 (d, J = 14.1 Hz, 1H, C_3-Ha), 2.91 (m, 1H, C_3-Hb), 2.66 (m, 2H, piperidin-CH_2), 2.47 (t, J = 10.5 Hz, 2H, piperidin-CH_2), 2.22 (t, J = 10.5 Hz, 1H, piperidin-4-CH), 1.74 - 2.11 (m, 4H, piperidin-CH_2); ^{13}CNMR (75 MHz, $CDCl_3$): δ 181.0, 167.9, 162.7 ($^1J_{CF}$ = 248.9 Hz), 161.8, 158.8 ($^1J_{CF}$ = 248.9 Hz), 150.8, 144.7, 129.3, 128.9, 126.02, 119.2, 114.2, 111.6, 104.3, 71.9, 62.4, 56.3, 55.3, 54.5, 53.4, 33.6, 29.7, 29.3; MS (ESI) m/z: 497.50 (M+H). HRMS (ESI) calcd for $C_{25}H_{26}F_2N_6O_3$ $[M+H]^+$ = 497.2034, found 497.2034.

2-(2,4-Difluorophenyl)-1-(4-(3-(furan-2-yl)-1,2,4-oxadiazol-5-yl)piperidin-1-yl)-3-(1*H*-1,2,4-triazol-1-yl)propan-2-ol (11c)

^{1}HNMR (300 MHz, $CDCl_3$) δ 8.08 (s, 1H, TriazC$_3$-H), 7.70 (s, 1H, TriazC$_5$-H), 7.51

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(m, 2H, furan-H), 7.02 (d, J = 3.4 Hz, 1H, Ar-H), 6.74 (m, 2H, Ar-H), 6.46 (m, 1H, furan-H), 5.03 (brs, 1H, OH), 4.46 (m, 2H, C_1-H), 3.02 (d, J = 13.6 Hz, 1H, C_3-Ha), 2.84 (m, 1H, C_3-Hb), 2.61 (d, J = 13.6 Hz, 2H, piperidin-CH_2), 2.39 (m, 2H, piperidin-CH_2), 2.14 (t, J = 11.1 Hz, 1H, piperidin-4-CH), 1.65 - 2.04 (m, 4H, piperidin-CH_2); ^{13}CNMR (75 MHz, $CDCl_3$): δ 181.4, 162.6 ($^1J_{CF}$ = 249.9 Hz), 161.0, 158.8 ($^1J_{CF}$ = 248.2 Hz), 150.9, 145.0, 144.6, 142.1, 129.3, 126.0, 113.5, 111.7, 111.5, 104.2, 72.1, 62.4, 56.1, 54.4, 53.3, 33.5, 29.6, 29.2; MS (ESI) m/z: 457.4 (M+H). HRMS (ESI) calcd for $C_{22}H_{22}F_2N_6O_3$ $[M+H]^+$ = 457.1712, found 457.1712.

2-(2,4-Difluorophenyl)-1-(4-(3-(thiophen-3-yl)-1,2,4-oxadiazol-5-yl)piperidin-1-yl)-3-(1*H*-1,2,4-triazol-1-yl)propan-2-ol (11d)

^{1}HNMR (300 MHz, $CDCl_3$) δ 8.15 (s, 1H, TriazC$_3$-H), 7.79 (s, 1H, TriazC$_5$-H), 7.75 (d, J = 3.5 Hz, 1H, thiophene-H), 7.57 (q, J = 7.5 Hz, 1H, thiophene-H), 7.49 (d, J = 4.9 Hz, 1H, thiophene-H), 7.14 (t, J = 4.0 Hz, 1H, Ar-H), 6.83 (m, 2H, Ar-H), 5.20 (br s, 1H, OH), 4.53 (m, 2H, C_1-H), 3.11 (m, 1H, C_3-Ha), 2.92 (m, 1H, C_3-Hb), 2.70 (m, 2H, piperidin-CH_2), 2.47 (m, 2H, piperidin-CH_2), 2.22 (m, 1H, piperidin-4-CH), 1.72 - 2.13 (m, 4H, piperidin-CH_2); ^{13}CNMR (75 MHz, $CDCl_3$): δ 181.2, 164.3, 162.7 ($^1J_{CF}$ = 248.2 Hz), 158.8 ($^1J_{CF}$ = 248.2 Hz), 151.0, 144.6, 129.4, 129.3, 129.2, 128.3, 127.9, 125.9, 111.6, 104.3, 72.1, 62.4, 56.2, 54.5, 53.4, 33.6, 29.6, 29.2; MS (ESI) m/z: 473.3(M+H). HRMS (ESI) calcd for $C_{22}H_{22}F_2N_6O_2S$ $[M+H]^+$ = 473.1522, found 473.1522.

2-(2,4-Difluorophenyl)-1-(4-(3-(thiophen-2-yl)-1,2,4-oxadiazol-5-yl)piperidin-1-yl)-3-(1*H*-1,2,4-triazol-1-yl)propan-2-ol (11e)

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^{1}HNMR (300 MHz, $CDCl_3$) δ 8.15 (s, 1H, TriazC_3-H), 8.01 (m, 1H, thiophene-H), 7.78 (s, 1H, TriazC_5-H), 7.56 (m, 2H, thiophene-H), 7.40 (m, 1H, Ar-H), 6.81 (m, 2H, Ar-H), 5.20 (brs, 1H, OH), 4.53 (m, 2H, C_1-H), 3.09 (d, J = 13.0 Hz, 1H, C_3-Ha), 2.90 (m, 1H, C_3-Hb), 2.67 (d, J = 13.3 Hz, 2H, piperidin-CH_2), 2.46 (m, 2H, piperidin-CH_2), 2.21 (t, J = 10.1 Hz, 1H, piperidin-4-CH), 1.79 - 2.09 (m, 4H, piperidin-CH_2); ^{13}CNMR (75 MHz, $CDCl_3$): δ 181.1, 164.6, 162.6 ($^1J_{CF}$ = 250.9 Hz), 158.8 ($^1J_{CF}$ = 245.6 Hz), 151.1, 144.7, 129.3, 128.2, 127.5, 127.0, 126.1, 126.0, 111.6, 104.3, 72.0, 62.4, 56.2, 54.5, 53.4, 33.5, 29.6, 29.2; MS (ESI) m/z: 473.3(M+H). HRMS (ESI) calcd for $C_{22}H_{22}F_2N_6O_2S$ $[M+H]^+$ = 473.1522, found 473.1522.

2-(2,4-Difluorophenyl)-1-(1*H*-1,2,4-triazol-1-yl)-3-(4-(3-(4-(trifluoromethyl)phenyl)-1,2,4-oxadiazol-5-yl)piperidin-1-yl)propan-2-ol (11f)

^{1}HNMR (300 MHz, $CDCl_3$) δ 8.17 (s, 1H, TriazC_3-H), 8.14 (s, 2H, Ar-H), 7.79 (s, 1H, TriazC_5-H), 7.72 (d, J = 8.1 Hz, 2H, Ar-H), 7.56 (m, 1H, Ar-H), 6.82 (m, 2H, Ar-H), 4.53 (m, 2H, C_1-H), 3.10 (d, J = 12.9 Hz, 1H, C_3-Ha), 2.94 (m, 1H, C_3-Hb), 2.68 (d, J = 13.6 Hz, 2H, piperidin-CH_2), 2.47 (m, 2H, piperidin-CH_2), 2.22 (t, J = 11.3 Hz, 1H, piperidin-4-CH), 1.79 - 2.10 (m, 4H, piperidin-CH_2); ^{13}CNMR (75 MHz, $CDCl_3$): δ 181.8, 174.6, 167.2, 162.6 ($^1J_{CF}$ = 249.9 Hz), 158.9 ($^1J_{CF}$ = 249.9 Hz), 151.1, 144.7, 132.8, 130.2, 129.3, 127.7, 126.0, 125.8, 125.5, 121.9, 111.6, 104.3, 72.0, 62.4, 56.2, 54.5, 53.4, 33.5, 29.7, 29.3; MS (ESI) m/z: 535.4(M+H). HRMS (ESI) calcd for $C_{25}H_{23}F_5N_6O_2$ $[M+H]^+$ = 535.1835, found 535.1835.

2-(2,4-Difluorophenyl)-1-(4-(3-(4-methoxyphenyl)-1,2,4-oxadiazol-5-yl)piperidin-1-yl)-3-(1*H*-1,2,4-triazol-1-yl)propan-2-ol (11g)

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^{1}HNMR (300 MHz, $CDCl_3$) δ 8.13 (s, 1H, TriazC$_3$-H), 7.76 (s, 1H, TriazC$_5$-H), 7.61 (d, *J* = 7.8 Hz, 1H, Ar-H), 7.54 (m, 2H, Ar-H), 7.34 (m, 1H, Ar-H), 7.00 (m, 1H, Ar-H), 6.83 (m, 2H, Ar-H), 4.50 (m, 2H, C_1-H), 3.83 (s, 3H, OCH_3), 3.07 (d, *J* = 13.4 Hz, 1H, C_3-Ha), 2.90 (m, 1H, C_3-Hb), 2.66 (d, *J* = 13.7 Hz, 2H, piperidin-CH_2), 2.45 (m, 2H, piperidin-CH_2), 2.20 (t, *J* = 11.3 Hz, 1H, piperidin-4-CH), 1.72 - 2.10 (m, 4H, piperidin-CH_2); MS (ESI) m/z: 497.4(M+H). ^{13}CNMR (75 MHz, $CDCl_3$): δ 181.2, 168.1, 162.6 ($^1J_{CF}$ = 249.5 Hz), 159.8, 158.9 ($^1J_{CF}$ = 247.0 Hz), 151.1, 144.7, 129.9, 129.6, 129.3, 127.9, 119.8, 117.6, 112.0, 111.7, 104.3, 72.0, 62.4, 56.2, 55.4, 54.5, 53.4, 33.5, 29.6, 29.2; HRMS (ESI) calcd for $C_{25}H_{26}F_2N_6O_3$ $[M+H]^+$ = 497.2019, found 497.2019.

2-(2,4-Difluorophenyl)-1-(4-(3-(p-tolyl)-1,2,4-oxadiazol-5-yl)piperidin-1-yl)-3-(1*H*-1,2,4-triazol-1-yl)propan-2-ol (11h)

^{1}HNMR (300 MHz, $CDCl_3$) δ 8.15 (s, 1H, TriazC$_3$-H), 7.92 (d, *J* = 8.1 Hz, 2H, Ar-H), 7.79 (s, 1H, TriazC$_5$-H), 7.56 (m, 1H, Ar-H), 7.28 (s, 1H, Ar-H), 7.25 (s, 1H, Ar-H), 6.82 (m, 2H, Ar-H), 4.50 (m, 2H, C_1-H), 3.11 (d, *J* = 13.4 Hz, 1H, C_3-Ha), 2.94 (d, *J* = 4.7 Hz, 1H, C_3-Hb), 2.68 (d, *J* = 13.2 Hz, 2H, piperidin-CH_2), 2.46 (m, 2H, piperidin-CH_2), 2.40 (s, 3H, CH_3), 2.22 (t, *J* = 11.0 Hz, 1H, piperidin-4-CH), 1.72 - 2.09 (m, 4H, piperidin-CH_2); ^{13}CNMR (75 MHz, $CDCl_3$): δ 181.1, 168.2, 162.7 ($^1J_{CF}$ = 247.9 Hz), 158.8 ($^1J_{CF}$ = 246.6 Hz), 151.1, 144.7, 141.4, 129.5, 129.3, 127.3, 126.1, 123.9, 111.6, 104.3, 72.0, 62.4, 56.2, 54.5, 53.4, 33.6, 29.7, 29.3, 21.5; MS (ESI) m/z: 481.4(M+H). HRMS (ESI) calcd for $C_{25}H_{26}F_2N_6O_2$ $[M+H]^+$ = 481.2136, found 481.2135.

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2-(2,4-Difluorophenyl)-1-(4-(3-(4-fluorophenyl)-1,2,4-oxadiazol-5-yl)piperidin-1-yl)-3-(1*H*-1,2,4-triazol-1-yl)propan-2-ol (11i)

^{1}HNMR (600 MHz, $CDCl_3$) δ 8.15 (s, 1H, TriazC_3-H), 8.04 (dd, *J* = 5.3 Hz, 8.7 Hz, 2H, Ar-H), 7.79 (s, 1H, TriazC_5-H), 7.56 (dd, *J* = 8.2 Hz, 15.4 Hz, 1H, Ar-H), 7.14 (t, *J* = 8.5 Hz, 2H, Ar-H), 6.83 (m, 2H, Ar-H), 4.56 (d, *J* = 14.1 Hz, 1H, C_1-Ha), 4.50 (d, *J* = 14.1 Hz, 1H, C_1-Hb), 3.11 (m, 1H, C_3-Ha), 2.93 (m, 1H, C_3-Hb), 2.69 (m, 2H, piperidin-CH_2), 2.47 (m, 2H, piperidin-CH_2), 2.22 (m, 1H, piperidin-4-CH), 2.05 (m,1H, piperidin-CH_2), 1.94 (m, 2H, piperidin-CH_2), 1.81 (m, 1H, piperidin-CH_2); MS (ESI) m/z: 485.4 (M+H). HRMS (ESI) calcd for $C_{24}H_{23}F_3N_6O_2$ $[M+H]^+$ = 485.1822, found 485.1822.

2-(2,4-Difluorophenyl)-1-(4-(3-(2-fluoro-4-(trifluoromethyl)phenyl)-1,2,4-oxadiazol-5-yl)piperidin-1-yl)-3-(1*H*-1,2,4-triazol-1-yl)propan-2-ol (11j)

^{1}HNMR (600 MHz, $CDCl_3$) δ 8.17 (t, *J* = 7.4 Hz, 1H, Ar-H), 8.14 (s, 1H, TriazC_3-H), 7.79 (s, 1H, TriazC_5-H), 7.57 (t, *J* = 8.4 Hz, 1H, Ar-H), 7.54 (d, *J* = 8.4 Hz, 1H, Ar-H), 7.50 (d, *J* = 10.2 Hz, 1H, Ar-H), 6.82 (m, 2H, Ar-H), 5.14 (br s, 1H, OH), 4.56 (d, *J* = 14.1 Hz, 1H, C_1-Ha), 4.51 (d, *J* = 14.1 Hz, 1H, C_1-Hb), 3.10 (d, *J* = 14.0 Hz, 1H, C_3-Ha), 2.97 (m, 1H, C_3-Hb), 2.70 (m, 2H, piperidin-CH_2), 2.48 (m, 2H, piperidin-CH_2), 2.22 (m, 1H, piperidin-4-CH), 1.79 - 2.09 (m, 4H, piperidin-CH_2); MS (ESI) m/z: 553.3 (M+H). HRMS (ESI) calcd for $C_{25}H_{22}F_6N_6O_2$ $[M+H]^+$ = 553.1712, found 553.1712.

In Vitro Antifungal Activity Assay

In vitro antifungal activity was measured according to the protocols from National

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Committee for Clinical Laboratory Standards (NCCLS) with a few modifications[26]. Serial dilution method in 96-well microtest plate was used to determine the minimum inhibitory concentration (MIC) of the target compounds. Briefly, for yeasts, the initial concentration of fungal suspension in RPMI 1640 medium was 10^3 CFU/ml. For filamentous fungi, the initial concentration of fungal suspension in RPMI 1640 medium was 5×10^3 CFU/ml. Tested compounds were dissolved in DMSO serially diluted in growth medium. The final concentrations of each compound ranged from 0.125 to 64 μg/mL. The yeasts were incubated at 35 °C and the filamentous fungi were incubated at 37 °C. Growth MIC was determined at 48 h after incubation. The growth inhibition was determined by spectrophotometer. Optical density was measured at 630 nm, and background optical densities were subtracted from that of each well. Each strain was tested in triplicate.The growth control wells contained 100 μl of the diluted fungal suspension. Sterility control wells contained 100 μl of RPMI 1640. The MICs at which 50% and 80% of the isolates were inhibited were determined (MIC_{50} and MIC_{80} respectively).

Hyphal Formation Assay

To evaluate the activity of the compounds **6g** and **11b** against yeast-to-hypha morphological transition, hyphal formation assay was carried out.[27] Briefly, *C. albicans* cells were harvested by centrifugation (3,000 rpm, 5 min) and washed three times with PBS. The *C. albicans* suspension was then adjusted to 1×10^6 cells/ml with Spider medium. The *C. albicans* suspension was divided into the wells in 12-well plates with different concentrations of **6g/11b** added. Then the 12-well plates

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were incubated in 37°C. After 3-h incubation, the cellular morphology was photographed.

Toxicity assay using *C. elegans*.

The assay was carried out as described previously[28] with slight modification. Synchronized *C. elegans* grown at 25°C for 3 days on NGM plates with OP50 as food. Adult worms were carefully collected and washed using sterile M9 buffer into a 15-ml conical tube. Thirty worms were then pipetted into a single well of a 12-well tissue culture plate containing 2 ml of liquid medium (80% M9 and 20% BHI). Six concentrations of **6g** and **11b** were tested, including 1, 5, 20, 40, 80, and 160 μg/ml. Worms were kept at 25°C and observed everyday to record dead worms. Two independent experiments were carried out. Photos were taken after incubation for 6 days.

Molecular Docking

Homology model of CACYP51 were obtained from our previous studies.[13] GOLD 4.1.2 as well as Goldscore function was used for molecular docking and the docking parameters were set as default.[29] The docking conformation was selected according to the docking score and visual inspection.

Acknowledgements

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References

1. D. A. Enoch, H. A. Ludlam and N. M. Brown, *J Med Microbiol*, 2006, 55,

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809-818.

2. C. C. Lai, C. K. Tan, Y. T. Huang, P. L. Shao and P. R. Hsueh, *J Infect Chemother*, 2008, 14, 77-85.
3. B. J. Park, K. A. Wannemuehler, B. J. Marston, N. Govender, P. G. Pappas and T. M. Chiller, *AIDS*, 2009, 23, 525-530.
4. Y. Aoyama, Y. Yoshida and R. Sato, *J Biol Chem*, 1984, 259, 1661-1666.
5. M. A. Pfaller, *Am J Med*, 2012, 125, S3-13.
6. R. Guillon, F. Pagniez, C. Rambaud, C. Picot, M. Duflos, C. Loge and P. Le Pape, *ChemMedChem*, 2011, 6, 1806-1815.
7. R. Guillon, F. Pagniez, F. Giraud, D. Crepin, C. Picot, M. Le Borgne, F. Morio, M. Duflos, C. Loge and P. Le Pape, *ChemMedChem*, 2011, 6, 816-825.
8. R. Guillon, C. Loge, F. Pagniez, V. Ferchaud-Roucher, M. Duflos, C. Picot and P. Le Pape, *J Enzyme Inhib Med Chem*, 2011, 26, 261-269.
9. R. Guillon, F. Giraud, C. Loge, M. Le Borgne, C. Picot, F. Pagniez and P. Le Pape, *Bioorg Med Chem Lett*, 2009, 19, 5833-5836.
10. F. Giraud, R. Guillon, C. Loge, F. Pagniez, C. Picot, M. Le Borgne and P. Le Pape, *Bioorg Med Chem Lett*, 2009, 19, 301-304.
11. O. Turel, *Expert Rev Anti Infect Ther*, 2011, 9, 325-338.
12. C. Sheng, Z. Miao, H. Ji, J. Yao, W. Wang, X. Che, G. Dong, J. Lu, W. Guo and W. Zhang, *Antimicrob Agents Chemother*, 2009, 53, 3487-3495.
13. C. Sheng, W. Wang, X. Che, G. Dong, S. Wang, H. Ji, Z. Miao, J. Yao and W. Zhang, *ChemMedChem*, 2010, 5, 390-397.
14. C. Sheng, W. Zhang, M. Zhang, Y. Song, H. Ji, J. Zhu, J. Yao, J. Yu, S. Yang, Y. Zhou and J. Lu, *J Biomol Struct Dyn*, 2004, 22, 91-99.
15. C. Sheng, S. Chen, H. Ji, G. Dong, X. Che, W. Wang, Z. Miao, J. Yao, J. Lu, W. Guo and W. Zhang, *J Mol Model*, 2010, 16, 279-284.
16. C. Sheng, W. Zhang, H. Ji, M. Zhang, Y. Song, H. Xu, J. Zhu, Z. Miao, Q. Jiang, J. Yao, Y. Zhou and J. Lu, *J Med Chem*, 2006, 49, 2512-2525.
17. Z. Jiang, J. Gu, C. Wang, S. Wang, N. Liu, Y. Jiang, G. Dong, Y. Wang, Y. Liu, J. Yao, Z. Miao, W. Zhang and C. Sheng, *Eur J Med Chem*, 2014, 82, 490-497.

View Article Online
DOI: 10.1039/C4MD00505H

18. C. Sheng, X. Che, W. Wang, S. Wang, Y. Cao, J. Yao, Z. Miao and W. Zhang, *Eur J Med Chem*, 2011, 46, 1706-1712.

19. S. Wu, Y. Zhang, X. He, X. Che, S. Wang, Y. Liu, Y. Jiang, N. Liu, G. Dong, J. Yao, Z. Miao, Y. Wang, W. Zhang and C. Sheng, *ChemMedChem*, 2014, 9, 2639-2646.

20. W. Wang, S. Wang, G. Dong, Y. Liu, Z. Guo, Z. Miao, J. Yao, W. Zhang and C. Sheng, *Med Chem Commun*, 2011, 2, 1066-1072.

21. C. Sheng and W. Zhang, *Curr Med Chem*, 2011, 18, 733-766.

22. J. Bostrom, A. Hogner, A. Llinas, E. Wellner and A. T. Plowright, *J Med Chem*, 2012, 55, 1817-1830.

23. B. S. Orlek, F. E. Blaney, F. Brown, M. S. Clark, M. S. Hadley, J. Hatcher, G. J. Riley, H. E. Rosenberg, H. J. Wadsworth and P. Wyman, *J Med Chem*, 1991, 34, 2726-2735.

24. M. Chayakulkeeree and J. R. Perfect, *Infect Dis Clin North Am*, 2006, 20, 507-544, v-vi.

25. T. R. Dagenais and N. P. Keller, *Clin Microbiol Rev*, 2009, 22, 447-465.

26. v. CLSI. 2008. Reference method for broth dilution antifungal susceptibility testing of yeasts, no. 14. Approved standard, 3rd ed. CLSI document M27-A3. Clinical and Laboratory Standards Institute, Wayne, PA.

27. M. M. Maidan, L. De Rop, J. Serneels, S. Exler, S. Rupp, H. Tournu, J. M. Thevelein and P. Van Dijck, *Mol Biol Cell*, 2005, 16, 1971-1986.

28. J. Breger, B. B. Fuchs, G. Aperis, T. I. Moy, F. M. Ausubel and E. Mylonakis, *PLoS Pathog*, 2007, 3, e18.

29. S. Wang, G. Jin, W. Wang, L. Zhu, Y. Zhang, G. Dong, Y. Liu, C. Zhuang, Z. Miao, J. Yao, W. Zhang and C. Sheng, *Eur J Med Chem*, 2012, 53, 292-299.

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Figure Legends

Figure 1. Chemical structures triazole antifungal agents.

Figure 2. Design rationale of the target compounds.

Figure 3. The binding mode of compounds **6g** (A) and **11b** (B) in the active site of CACYP51.

Figure 4. Effects of different concentrations of compounds **6g** and **11b** on hyphal formation with FCZ as a positive agent. Exponentially growing *C. albicans* SC5314 cells were transferred to hypha-inducing Spider liquid media. The cellular morphology was photographed after incubation at 37°C for 3 h. Scale bar = 100 μm.

Figure 5. Toxicity test using a *C. elegans* model. Six concentrations: 1, 5, 20, 40, 80, 160 μg/ml were tested. Almost all the worms even the highest concentration 160 μg/ml survived indicating no relevant toxicity of **6g** and **11b** shown in *C. elegans* model.

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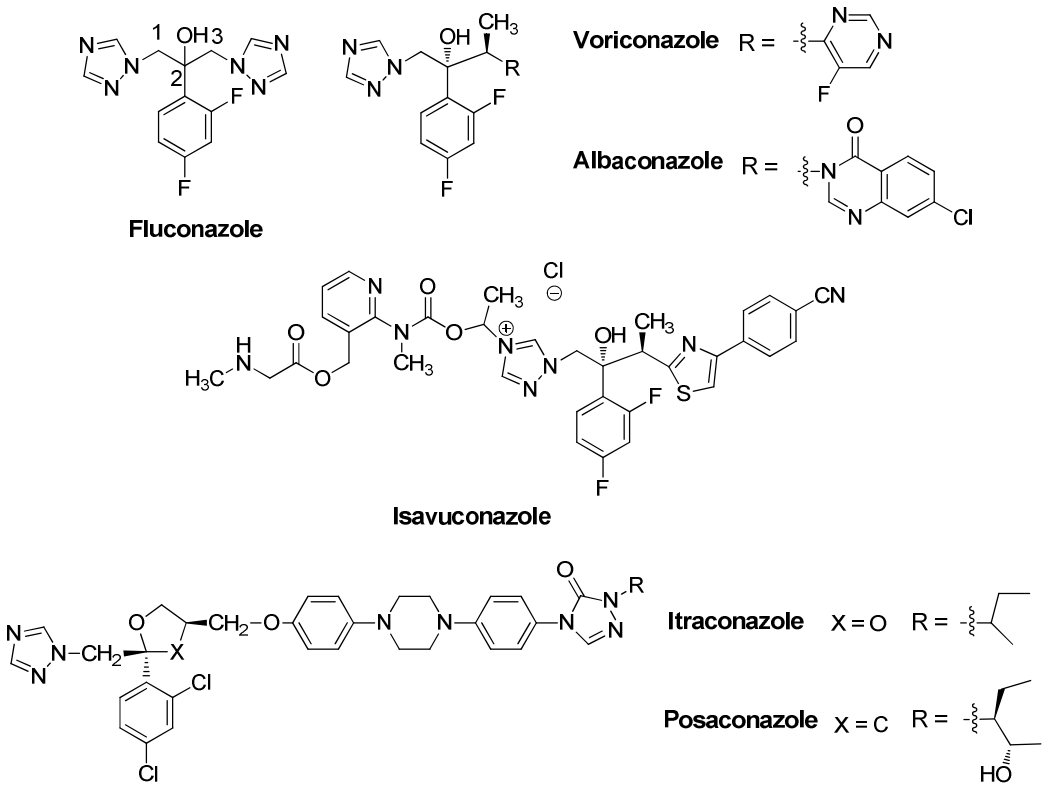

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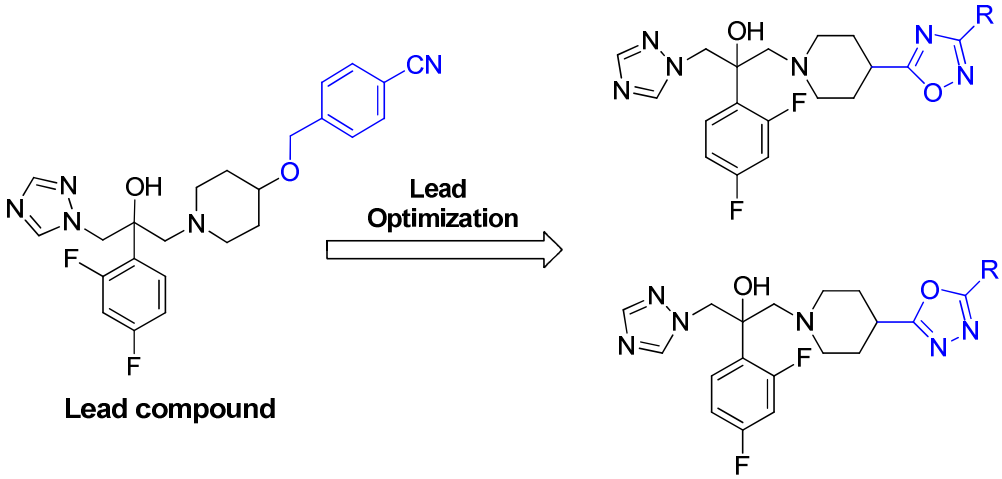

Figure 2. Design rationale of the target compounds.

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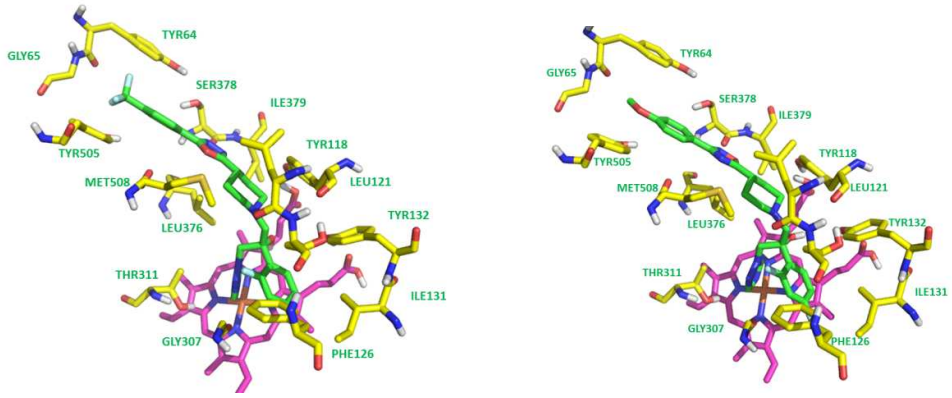

Figure 3. The binding mode of compounds **6g** (A) and **11b** (B) in the active site of CACYP51.

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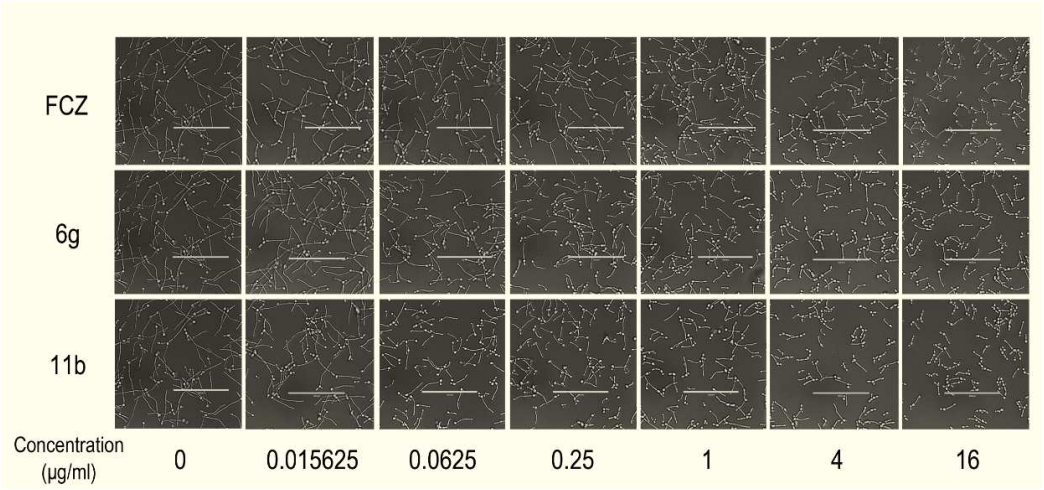

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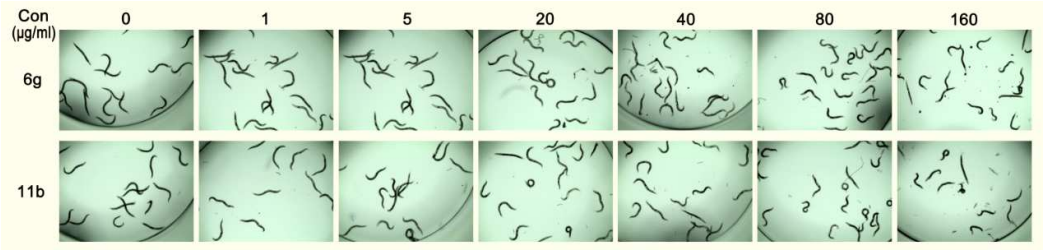

Figure 5. Toxicity test using a *C. elegans* model. Six concentrations: 1, 5, 20, 40, 80, 160 μg/ml were tested. Almost all the worms even the highest concentration 160 μg/ml survived indicating no relevant toxicity of **6g** and **11b** shown in *C. elegans* model.

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Table 1. *In vitro* antifungal activities of the target compounds (MIC_{80}, μg/mL)

Comd.	*C. alb.*	*C. par.*	*C. neo.*	*C. gla.*	*A. fum.*	*T. rub*	*M. gyp*
6a	0.125	1	0.5	4	32	4	4
6b	0.125	0.5	0.25	2	8	1	1
6c	0.063	0.5	≤0.125	0.5	8	2	2
6d	0.125	0.5	≤0.125	1	16	2	2
6e	0.125	4	4	8	>64	16	16
6f	0.063	1	0.5	2	4	1	1
6g	0.031	0.5	0.25	2	8	1	1
6h	0.063	0.5	≤0.125	1	16	2	2
6i	0.25	2	1	2	16	2	0.5
6j	0.5	4	1	8	>64	8	64
6k	0.5	2	2	4	16	4	4
6l	2	16	4	16	>64	32	4
6m	1	8	4	8	32	4	8
6n	0.125	0.5	≤0.125	0.5	16	2	4
6o	0.25	2	1	4	32	2	2
11a	0.063	0.25	0.125	0.125	2	1	0.5
11b	0.016	0.25	0.25	1	2	0.5	2
11c	0.063	1	0.5	1	16	2	2
11d	0.5	4	2	4	32	4	4
11e	0.063	2	1	2	8	1	2
11f	0.5	4	2	2	32	4	2
11g	1	4	2	4	32	4	4
11h	0.5	2	4	4	16	2	8
11i	0.5	2	1	2	16	4	1
11j	1	2	2	4	16	1	0.5
FCZ	0.25	2	0.25	4	>64	2	32

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

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Table 2. MICs of compounds **6g** and **11b** against fluconazole-resistant *C. albicans* strains.

C. albicans	**MIC_{50} (μg/mL)**			**MIC_{80} (μg/mL)**		
strain	**FLC**	**6g**	**11b**	**FLC**	**6g**	**11b**
103	256	0.25	0.25	>1024	8	4
805	256	0.0625	0.125	>1024	16	8
0710922	512	0.125	0.125	>1024	16	16

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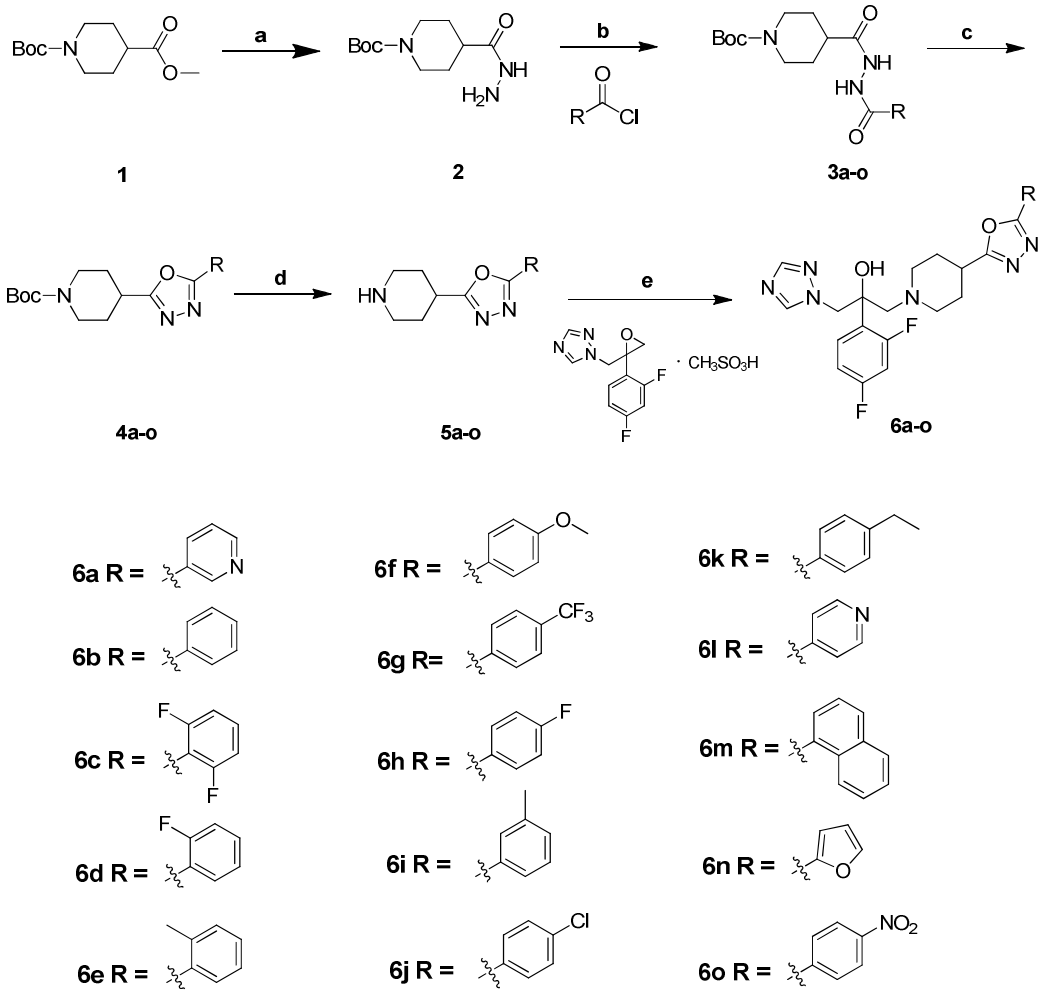

Scheme 1. Reagents and conditions:**:** a. $NH_2NH_2{\cdot}H_2O$, EtOH, RT, 12h, 95.0%; b. TEA, DCM, RT, 2h, 24%-100%; c. Et_3N, TsCl, THF, 78℃, 10h, 23%-100%; d. TFA, CH_3Cl_2, 10h, 78%-100%; e. Et_3N, EtOH, 78℃, 5h, 31%-82%.

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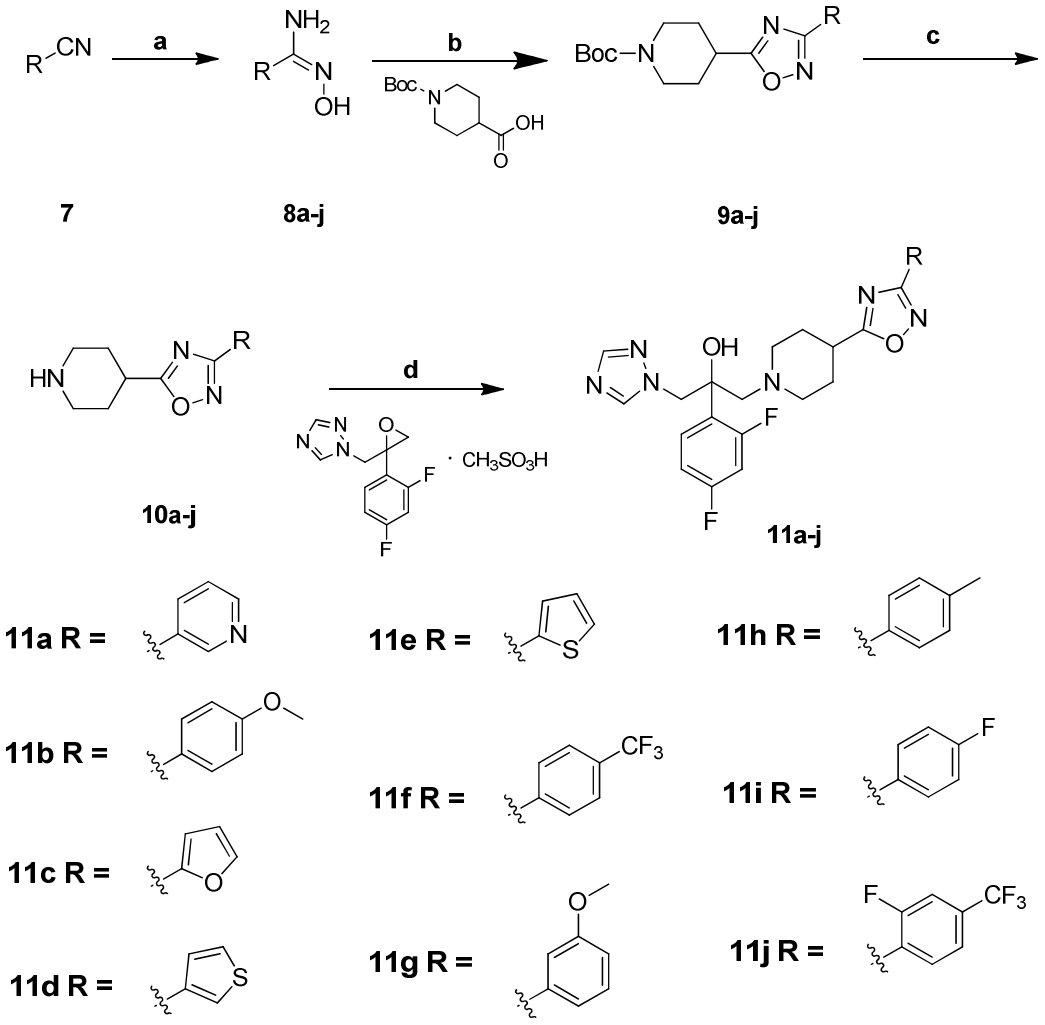

Scheme 2. Reagents and conditions: a. $NH_2OH{\cdot}HCl$, EtOH, 78℃, 8h, 54%-100%; b. 1-(*tert*-Butoxycarbonyl)isonipecotic acid, EDCI, DIPEA, HOBt, dioxane, 100℃, 10h, 20%-46%; c. TFA, CH_3Cl_2, 10h, 56%-100%; d. Et_3N, EtOH, 78℃, 5h, 37%-74%.

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Graphical Abstract

Discovery of Highly Potent Triazole Antifungal Agents with Piperdine-oxadiazole Side Chains

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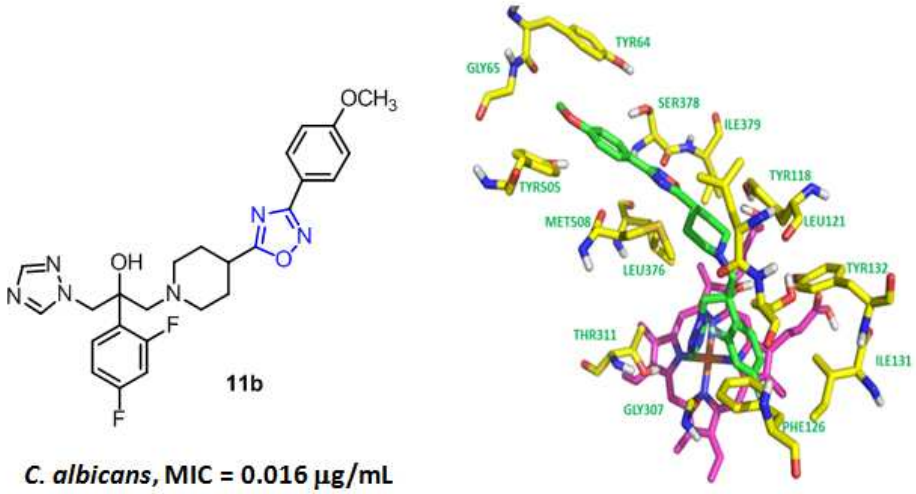

A series of novel triazole antifungal agents containing piperdine-oxadiazoleside chains were designed and synthesized. Compounds **11b** was highly active against *Candida albicans* with a MIC value of 0.016 μg/mL.