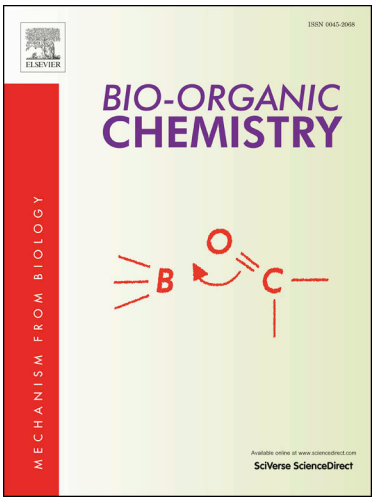
Journal Pre-proofs




Short communication

# Design, synthesis, and *in vitro* evaluation of novel triazole analogues featuring isoxazole moieties as antifungal agents

Fei Xie, Tingjunhong Ni, Zichao Ding, Yumeng Hao, Ruina Wang, Ruilian Wang, Ting Wang, Xiaoyun Chai, Shichong Yu, Yongsheng Jin, Yuanying Jiang, Dazhi Zhang

PII: S0045-2068(20)31279-7
DOI: https://doi.org/10.1016/j.bioorg.2020.103982
Reference: YBIOO 103982

To appear in: *Bioorganic Chemistry*

Received Date: 3 December 2019
Revised Date: 23 May 2020
Accepted Date: 29 May 2020

Please cite this article as: F. Xie, T. Ni, Z. Ding, Y. Hao, R. Wang, R. Wang, T. Wang, X. Chai, S. Yu, Y. Jin, Y. Jiang, D. Zhang, Design, synthesis, and *in vitro* evaluation of novel triazole analogues featuring isoxazole moieties as antifungal agents, *Bioorganic Chemistry* (2020), doi: https://doi.org/10.1016/j.bioorg.2020.103982

# Design, synthesis, and *in vitro* evaluation of novel triazole analogues featuring isoxazole moieties as antifungal agents

Fei Xie[a,†], Tingjunhong Ni[b,†], Zichao Ding[a,†], Yumeng Hao[a], Ruina Wang[c], Ruilian Wang[c], Ting Wang[a], Xiaoyun Chai[a], Shichong Yu[a], Yongsheng Jin[a], Yuanying Jiang[b,*], Dazhi Zhang[a,*]

[a] *Department of Organic Chemistry, School of Pharmacy, Second Military Medical University, 325 Guohe Road, Shanghai 200433, China*

[b] *Shanghai Tenth People's Hospital, Tongji University School of Medicine, 301 Middle Yanchang Road, Shanghai 200072, China*

[c] *Center of New Drug research, School of Pharmacy, Second Military Medical University, 325 Guohe Road, Shanghai 200433, China*

[†] These authors contributed equally to this work.

* Corresponding Author

Jiang Y. Tel.: +86-021-81871357, E-mail address: 13761571578@163.com

Zhang D. Tel.: +86-021-81871226, E-mail address: zdzhang_yjhx@smmu.edu.cn

**Abstract**: In order to develop novel antifungal agents, based on our previous work, a series of (2*R*,3*R*)-3-((3-substitutied-isoxazol-5-yl)methoxy)-2-(2,4-difluorophenyl)-1-(1*H*-1,2,4-triazol-1-yl) butan-2-ol (**a1**-**a26**) were designed and synthesized. All of the compounds exhibited good *in vitro* antifungal activities against eight human pathogenic fungi. Among them, compound **a6** showed excellent inhibitory activity against *Candida albicans* and *Candida parasilosis* with $MIC_{80}$ values of 0.0313 μg/mL. In addition, compounds **a6**, **a9**, **a12**, **a13** and **a14** exhibited moderate inhibitory activities against fluconazole-resistant isolates with $MIC_{80}$ values ranging from 8 μg/mL to 16 μg/mL. Furthermore, compounds **a6**, **a12** and **a23** exhibited low inhibition profiles for CYP3A4. Clear SARs were analyzed, and the molecular docking experiment was carried out to further investigate the relationship between **a6** and the target enzyme CYP51.

**Keywords**: Triazole; Antifungal activity; Structure-activity relationship; Synthesis

## 1. Introduction

During the last three decades, invasive fungal infections (IFIs) continue to pose a serious threat to human health, especially among people with HIV infection, organ transplant, cancer and autoimmune diseases[1]. More than 90% of all reported fungal-related deaths mainly result from three genera: candidiasis (*Candida albicans*, mortality: 46%-75%), cryptococcosis (*Cryptococcus neoformans*, mortality: 20%-70%), and aspergillosis (*Aspergillus fumigates*, mortality: 30%-90%)[2]. Clinically, treatment for IFIs contains three major classes of drugs which include azoles, polyenes and echinocandins[3]. However, with the continuous emergence of drug resistance and the undesired side effects on existing available antifungal drugs, new effective agents with improved profiles are urgently needed[4].

Triazoles are the most used antifungal agents due to their high therapeutic index. Since fluconazole was launched in 1988, followed by itraconazole (1989), voriconazole (2002), posaconazole (2005) and isavuconazole (2015) (**Fig.1**), more than 20 antifungal "conazoles" have been approved by the FDA in the past 50 years. They target and act by inhibiting the CYP51 (cytochrome P450 14α-demethylase), a necessary enzyme in the biosynthesis of ergosterol[5]. SAR studies[6-8] of these antifungal triazoles revealed a common pharmacophore which contained a triazole ring linked to a dihalophenyl ring through a two-carbon chain, and the carbon alpha to the phenyl ring bore a hydroxyl group. The difference between fluconazole and other azoles mainly lied in the optimization of the side chains attached to the pharmacophore.

In our previous work[9] (**Fig.1**), isoxazole moiety was introduced into the side chain by replacing the thiazole ring of ravuconazole and isavuconazole, respectively. Results indicated that 2,4-difluorophenyl is better than 2,5-difluorophenyl, and most of the designed compounds exhibited potent *in vitro* antifungal activities against nearly all tested fungi. The introduction of isoxazole may contribute to the increased efficacy and decreased toxicity, improving some pharmacokinetics profiles[10-12]. In this paper, we retained the isoxazole and 2,4-difluorophenyl moieties and meanwhile extended the side chain properly by adding an ether bond for better interaction with CYP51 channel residues. Herein, a series of isoxazole methoxyl motif containing triazoles were rationally designed and synthesized, and their *in vitro* antifungal activities were evaluated.

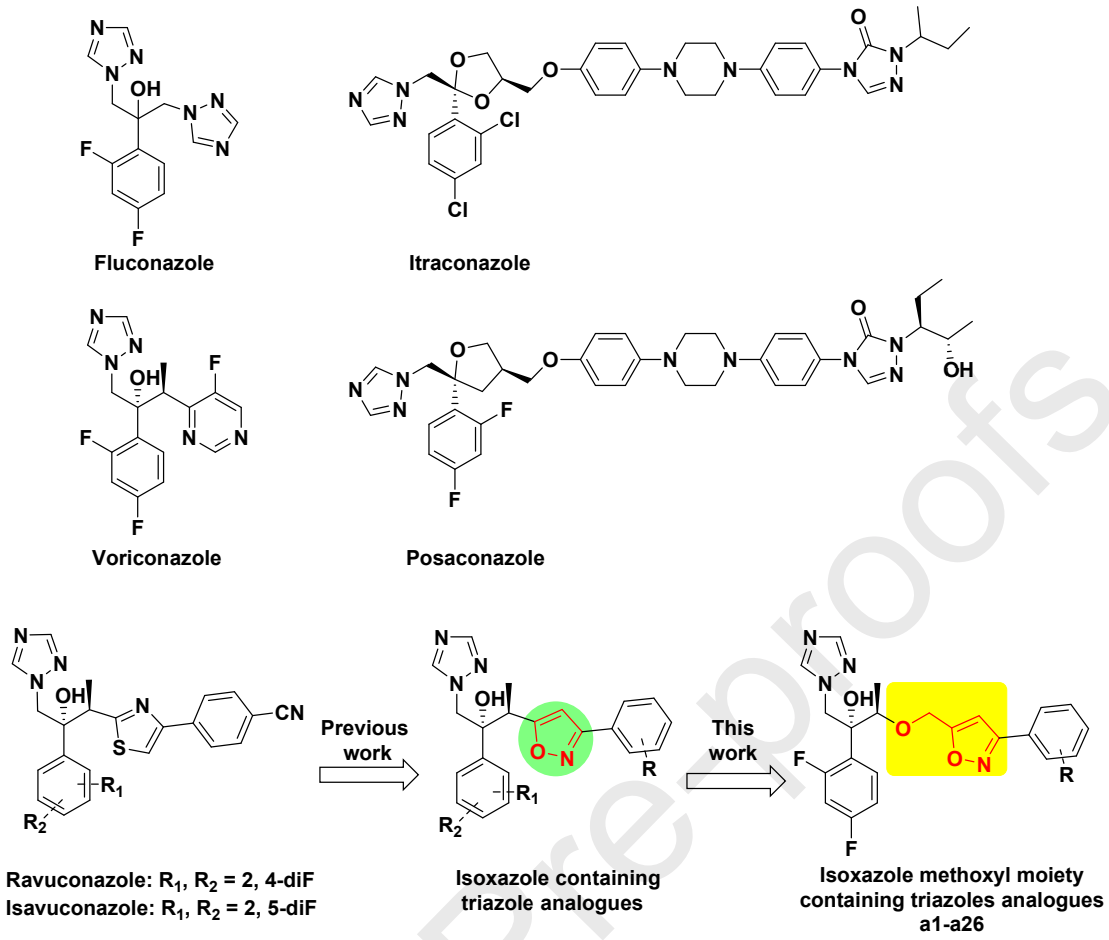
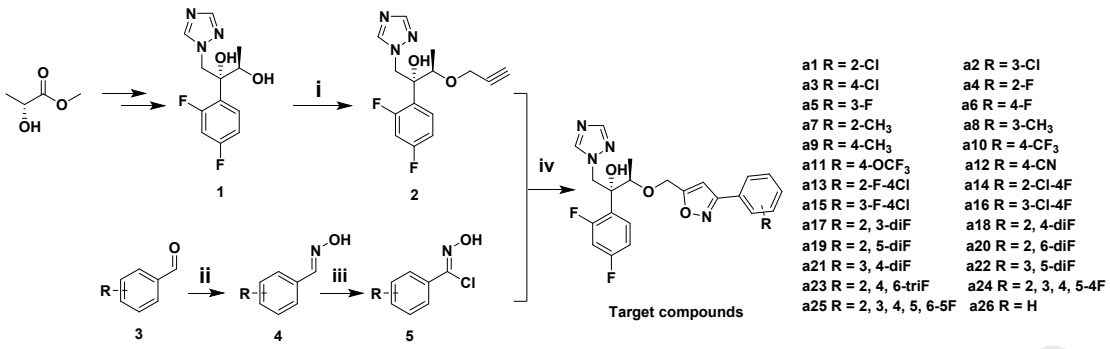



**Fig.1**. Structures of triazole antifungal agents and the designed compounds.

## 2. Chemistry

The general synthetic methodology for the preparation of the target compounds (**a1-a26**) is depicted in **Scheme 1**. As a key intermediate of our designed compounds, the diol **1** was synthesized according to the literature[13]. X-ray crystallographic analysis clearly supports the chiral structure of alkyne **2** to construct **a1**–**a26** (**Fig.2**, **Table S1** in the supplementary material). The intermediates **5** were prepared by the chlorination of benzaldehyde oximes **4** which were obtained by the hydroxylamination of various substituted benzaldehydes **3**. Then, intermediates **5** were reacted with alkyne **2** by the 1,3-dipolar cycloaddition to form **a1-a26**. Details of all procedure and data are described in supporting information.




**Scheme 1**. Reagents and conditions: (i) 3-bromoprop-1-yne, $Cs_2CO_3$, $CH_3CN$, 80℃, 12 h; (ii) $NH_2OH{\cdot}HCl$, $NaHCO_3$, $H_2O$, methanol, r.t., 2 h; (iii) *N*-chlorosuccinimide, DMF, 35℃, 2 h; (iv) $Et_3N$, $ZnCl_2$, THF, 35℃, 16-20 h.

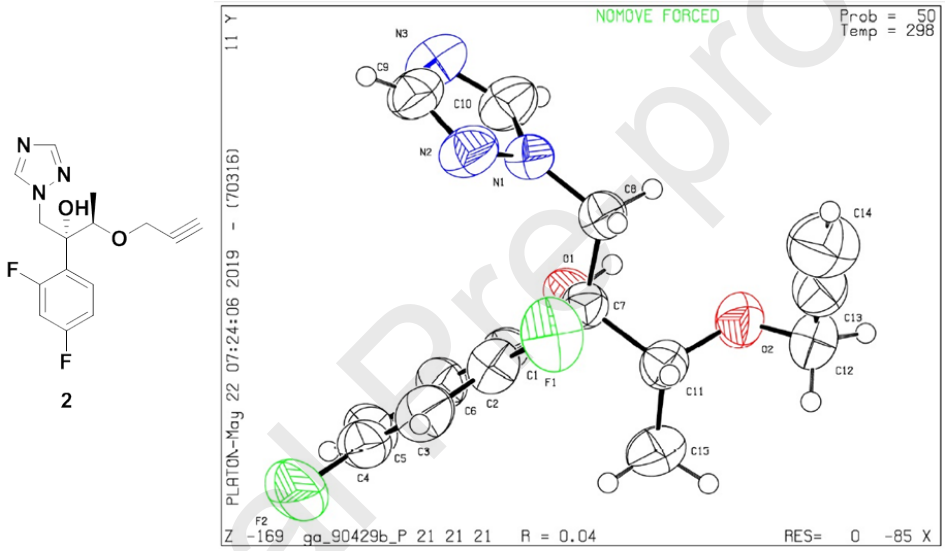



**Fig.2**. Single-crystal structure of alkyne **2**.

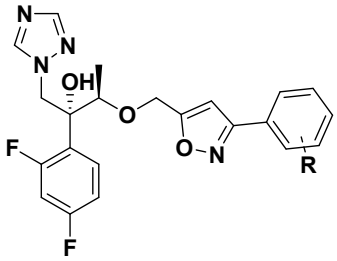
## 3. Pharmacology

The *in vitro* antifungal activities of **a1-a26** were evaluated against eight human pathogenic fungi inculding *Candida albicans*, *Candida parapsilosis*, *Cryptococcus neoformans*, *Candida glabrata*, *Aspergillus fumigatus*, *Trichophyton rubrum* and *Microsporum gypseum*. The minimum inhibitory concentration (MIC) determination was measured by serial dilution method in 96-well microtest plates with RPMI 1640 as the test medium according to the protocols recommended by National Committee for Clinical Laboratory Standards (NCCLS, USA)[14]. The $MIC_{80}$ was defined as the first well with an approximate 80% reduction in growth compared with the drug-free well. For assays, the target compounds to be tested were dissolved in dimethyl sulfoxide (DMSO), serially diluted in growth medium, inoculated and incubated at 35 °C. Growth $MIC_{80}$ was determined at 24 h for *C. albicans*, at 72 h for *C. neoformans*, and at 5-7 days for three

filamentous fungi. Fluconazole (FCZ) and ravuconazole (RCZ) served as positive controls. The results of assays are summarized in **Table 1** and **Table 2**. All of our susceptibility tests were evaluated twice by each antifungal agent.

Compounds **a6**, **a12**, **a23** were tested to assess *in vitro* potency in CYP3A4 enzyme assays. The 50% inhibitory concentration ($IC_{50}$) values for CYP3A4 enzyme were determined in human hepatocyte microsomes using 80 μM testosterone as substrate (a concentration equal to the Km determined in the same lab). Reactions were analyzed for products using LC-MS/MS methods, and $IC_{50}$ values (in μM) were determined by fitting a 4-parameter logistical fit to the dose response data. The results of assays are summarized in **Table 3**. All of our tests were evaluated twice by each antifungal agent.

**Table 1** *In vitro* antifungal activities of the target compounds against fluconazole-susceptible pathogenic fungi.




| *Compd* | R | MIC$_{80}$ (μg/mL) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | *C.alb* Y0109 | *C.alb* SC5314 | *C.par* 22019 | *C.gla* 537 | *C.neo* 32609 | *A.fum* 7544 | *T.rub cmccftla* | *M.gyp cmccftla* |
| **a1** | 2-Cl | 0.125 | 0.125 | 0.125 | 0.25 | 1 | 8 | 2 | 2 |
| **a2** | 3-Cl | 0.125 | 0.125 | 0.125 | 0.25 | 2 | 4 | 1 | 1 |
| **a3** | 4-Cl | 0.125 | 0.125 | 0.125 | 0.25 | 1 | 4 | 2 | 2 |
| **a4** | 2-F | 0.0625 | 0.0625 | 0.0625 | 0.125 | 1 | 4 | 2 | 1 |
| **a5** | 3-F | 0.0313 | 0.0625 | 0.0625 | 0.125 | 0.5 | 4 | 1 | 1 |
| **a6** | 4-F | 0.0313 | 0.0625 | 0.0313 | 0.125 | 0.5 | 4 | 1 | 0.5 |
| **a7** | 2-$CH_3$ | 0.0313 | 0.0625 | 0.125 | 0.25 | 1 | 4 | 4 | 2 |
| **a8** | 3-$CH_3$ | 0.125 | 0.25 | 0.25 | 0.5 | 2 | 8 | 4 | 2 |
| **a9** | 4-$CH_3$ | 0.0313 | 0.0625 | 0.125 | 0.125 | 0.5 | 4 | 2 | 1 |
| **a10** | 4-$CF_3$ | 0.125 | 0.25 | 0.25 | 0.25 | 1 | 4 | 1 | 1 |
| **a11** | 4-$OCF_3$ | 0.125 | 0.5 | 0.5 | 0.5 | 1 | 4 | 1 | 1 |
| **a12** | 4-CN | 0.0313 | 0.0625 | 0.125 | 0.125 | 0.5 | 4 | 1 | 0.5 |
| **a13** | 2-F-4-Cl | 0.25 | 0.5 | 0.5 | 0.5 | 2 | 4 | 2 | 1 |
| **a14** | 2-Cl-4-F | 0.125 | 0.25 | 0.25 | 0.5 | 1 | 16 | 2 | 1 |
| **a15** | 3-F-4-Cl | 0.125 | 0.5 | 0.25 | 0.25 | 2 | 16 | 1 | 1 |
| **a16** | 3-Cl-4-F | 0.5 | 0.5 | 0.5 | 0.5 | 1 | 16 | 1 | 2 |
| **a17** | 2,3-diF | 0.25 | 0.25 | 0.5 | 0.5 | 1 | 8 | 2 | 1 |
| **a18** | 2,4-diF | 0.125 | 0.25 | 0.25 | 0.5 | 1 | 8 | 1 | 2 |
| **a19** | 2,5-diF | 0.125 | 0.125 | 0.25 | 0.5 | 2 | 8 | 2 | 1 |
| **a20** | 2,6-diF | 0.125 | 0.125 | 0.25 | 0.25 | 2 | 16 | 2 | 1 |
| **a21** | 3,4-diF | 0.25 | 0.125 | 0.25 | 0.5 | 2 | 16 | 1 | 2 |
| **a22** | 3,5-diF | 0.25 | 0.25 | 0.5 | 0.25 | 2 | 8 | 1 | 2 |
| **a23** | 2,4,6-triF | 0.0625 | 0.125 | 0.125 | 0.125 | 1 | 4 | 2 | 1 |
| **a24** | 2,3,4,5-4F | 0.0625 | 0.125 | 0.125 | 0.25 | 1 | 8 | 2 | 1 |
| **a25** | 2,3,4,5,6-5F | 0.0625 | 0.125 | 0.125 | 0.25 | 1 | 8 | 2 | 1 |
| **a26** | H | 0.0313 | 0.0625 | 0.25 | 0.25 | 1 | 8 | 2 | 2 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **FCZ** | / | 0.25 | 0.5 | 1 | 2 | 1 | >64 | 8 | 32 |
| **RCZ** | / | 0.0625 | 0.0625 | 0.25 | 0.125 | 0.125 | 2 | 0.125 | 1 |

Abbreviations: *C.alb*, *Candida albicans*; *C.par*, *Candida parasilosis*; *C.gla*, *Candida glabrata*; *C.neo*, *Cryptococcus neoformans*; *A.fumi*, *Aspergillus fumigatus*; *T.rub*, *Trichophyton rubrum*; *M.gyp*, *Microsporumgypseum*; FCZ, Fluconazole; RCZ, Ravuconazole.

**Table 2** *In vitro* antifungal activities of the target compounds against fluconazole-resistant *Candida albicans*.

| | | **$MIC_{80}$ (μg/mL)** | |
|---|---|---|---|
| ***Compd*** | **R** | *C.alb* 100 | *C.alb* 103 |
| **a6** | 4-F | 8 | 16 |
| **a9** | 4-$CH_3$ | 16 | 16 |
| **a11** | 4-$OCF_3$ | >64 | >64 |
| **a12** | 4-CN | 8 | 8 |
| **a13** | 2-F-4-Cl | 8 | 8 |
| **a14** | 2-Cl-4-F | 8 | 16 |
| **FCZ** | / | >64 | >64 |

Abbreviations: *C.alb*, *Candida albicans*; FCZ, Fluconazole.

**Table 3** *In vitro* inhibition of CYP3A4 by representative compounds.

| ***Compd*** | ***C.alb* SC5314 $MIC_{80}$ (μg/mL)** | **CYP3A4 $IC_{50}$ (μM)[a]** | **Selectivity index[b]** |
|---|---|---|---|
| **a6** | 0.0625 | 1.18 | 18.88 |
| **a12** | 0.0625 | 2.49 | 39.84 |
| **a23** | 0.125 | 1.10 | 8.80 |
| **RCZ** | 0.0625 | 1.01 | 16.16 |
| **KCZ** | 0.125 | 0.0125 | 0.10 |

Abbreviations: *C.alb*, *Candida albicans*; RCZ, Ravuconazole; KCZ, Ketoconazole.

[a.] Inhibition of CYP3A4 measured in microsomes obtained from pooled human hepatocytes.

[b.] *In vitro* selectivity calculated as CYP3A4 $IC_{50}$ / *C.alb* SC5314 $MIC_{80}$.

## 4. Results and discussion

As shown in **Table 1**, all the target compounds showed good or moderate activities against the eight tested fungi compared with positive controls. The $MIC_{80}$ values against *C.alb* Y0109 and *C.alb* SC5314 are ranging from 0.0313 μg/mL to 0.5 μg/mL, thus indicating the antifungal activities of these compounds were comparable or superior to FCZ and RCZ. For example, compounds **a5**, **a6**, **a7**, **a9**, **a12**, and **a26** exhibited $MIC_{80}$ values of 0.0313 μg/mL against *C.alb* Y0109. This concentration is lower than the $MIC_{80}$ values of FCZ ($MIC_{80}$ = 0.25 μg/mL) and RCZ

($MIC_{80}$ = 0.0625 μg/mL). On the *C.par* 22019 strains, except for compounds **a11**, **a13**, **a16**, **a17** and **a22** ($MIC_{80}$ = 0.5 μg/mL), the $MIC_{80}$ values of our compounds are equal to or lower than RCZ ($MIC_{80}$ = 0.25 μg/mL). Most of the compounds including fluconazole only showed moderate activities against *C.gla* 537, *C.neo* 32609*, T.rub* and *M.gyp*. Among them, on the *M.gyp* strain, compounds **a6** and **a12** with the $MIC_{80}$ values of 0.5 μg/mL are 64-fold more potent than FCZ ($MIC_{80}$ = 32 μg/mL) and comparable to RCZ ($MIC_{80}$ = 1 μg/mL). FCZ is inactive against *A.fumi* 7544, while our compounds showed moderate activities ($MIC_{80}$ range: 4 μg/mL to 16 μg/mL).

Based on the antifungal activities data, preliminary SARs of these novel azoles were obtained. In compounds **a1**-**a9**, we systematically synthesized derivatives with the *ortho*, *meta* and *para* mono-substituted R groups (R = Cl, F, $CH_3$, respectively). Compounds with F-substituted (**a4**-**a6**) exhibited better antifungal activities against *C.alb* Y0109, *C.alb* SC5314 and *C.par* 22019 ($MIC_{80}$ range: 0.0313 μg/mL to 0.0625 μg/mL) than compounds with Cl-substituted (**a1**-**a3**) ($MIC_{80}$ = 0.125 μg/mL). $CH_3$-substituted compounds (**a7**-**a9**) apart from **a8** (m-$CH_3$) showed better antifungal activities against all the tested fungi compared with RCZ. These results indicate that the introduction of mono-F and *ortho*-$CH_3$, *para*-$CH_3$ to the terminal phenyl group contributes to the improvement of antifungal effect. Compounds **a10**-**a12**, **a12** (*p*-CN) displayed better antifungal activities against six pathogens (*C.alb* Y0109, *C.alb* SC5314 *C.par* 22019, *C.gla* 537, *C.neo* 32609 and *M.gyp*) than **a10** (*p*-$CF_3$), **a11** (*p*-$OCF_3$). On the other hand, different from our previous results, increasing the number of fluorine atoms substituted on the phenyl ring (**a13**-**a25**) had less effect on the improvement of antifungal activity. This point may attribute to the extension of the side chain, so that the fluorine atom on the phenyl ring cannot form a hydrogen bond with surrounding amino acid residues.

As more and more serious azole-resistance problems are emerging in clinic, it is also worth further evaluating these novel synthesized compounds against fluconazole-resistant species of *Candida albicans*. The inhibitory activities of six representative compounds, **a6**, **a9**, **a11**, **a12**, **a13** and **a14**, were tested. Results are presented as $MIC_{80}$ values in **Table 2**. The $MIC_{80}$ values of FCZ against *C. albicans* 100 and *C. albicans* 103 were >64 μg/mL. Except compound **a11**, the remaining compounds exhibited moderate activities ($MIC_{80}$ range: 8 μg/mL to 16 μg/mL).

Considering that triazole antifungal agents target fungal CYP51 protein and inhibit human CYP enzymes, resulting in hepatotoxicity as a side effect, three representative compounds, **a6**,

**a12**, and **a23**, were tested for their inhibition of CYP3A4 in human hepatocytes microsomes. As shown in **Table 3**, the $IC_{50}$ of **a6**, **a12**, and **a23** was 1.18 μM, 2.49 μM and 1.10 μM, respectively. Selectivity index (SI) can indicate the safety of a compound to some extent[15]. The *in vitro* safety profiles of them were superior to marketed azoles such as ketoconazole (CYP3A4 $IC_{50}$ = 0.0125 μM, SI = 0.10) and comparable to ravuconazole (CYP3A4 $IC_{50}$ = 1.01 μM, SI = 16.16).

In order to further investigate the interactions between the novel compounds and CYP51, a molecular docking study was carried out. 2,4-difluorophenylisoxazole containing triazole (compound **a'14** in our previous work， **Figure S1** in the supplementary material) and **a6** were docked into the active site of CYP51 (PDB ID: 5TZ1) using the SYBYL-X 2.0. As shown in **Fig.3 (A)**, the triazoly group, hydroxyl group and difluorophenyl group of **a'14** were considered as forming nonbonding interactions with Hem601, Phe126, Thr311, Tyr132, and Thr122. The isoxazole moiety as the side chain extended into CYP51 channel to form hydrophobic and van der Waals interactions with surrounding residues Leu121 and Leu376, while the terminal 2,4-difluorophenyl group interacted with Met508, Phe380 and Phe233. Especially, due to the length of side chain, its 2-fluorine atom forms a hydrogen bond with Tyr-118. In the case of **a6** showed in **Fig.3 (B)**, it shared a similar binding mode with **a'14** in the active site. The introduced methoxyl moiety appropriately stretched the length of side chain into CYP51 channel. Moreover, unlike the ways that **a'14** forms hydrogen bond, the oxygen atom of isoxazole moiety was found to form a hydrogen bond with Tyr118 in the distance of 2.1 Å.

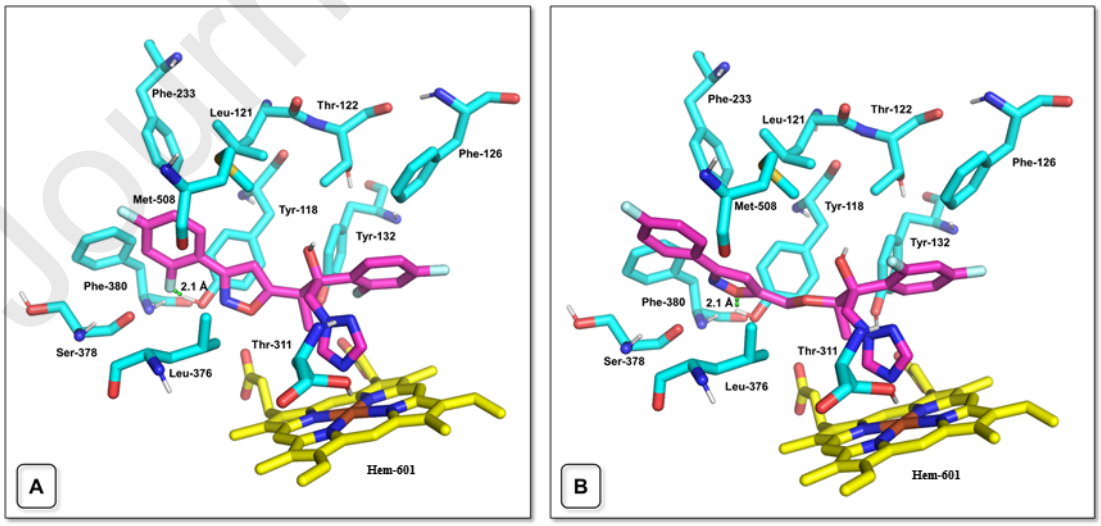



**Fig.3.** 3D presentation of docked triazoles in the active site of CYP51.

(A) The binding mode of compound **a'14**. (B) The binding mode of compound **a6**

## 5. Conclusion

In summary, based on our previous work, we further designed and synthesized a series of triazole analogues by replacing 4-cyanophenylthioazole in ravuconazole with substituted phenylisoxazole methoxyls. Most of the designed compounds exhibited moderate *in vitro* antifungal activities against eight fungal isolates. Among them, compound **a6** showed excellent inhibitory activity against *Candida albicans* and *Candida parasilosis* with $MIC_{80}$ values of 0.0313 μg/mL. Moreover, compounds **a6**, **a9**, **a12**, **a13** and **a14** were also tested against fluconazole-resistant isolates, suggesting that the appropriate derivation on azole drugs might be a useful approach to combat fungal resistant problem. SARs study demonstrates that mono-F to the terminal phenyl ring is favorable to the antifungal activity. Docking study indicates that the methoxyl motif forms a flexible side chain extending into CYP51 channel and supports the oxygen atom of isoxazole to generate a key hydrogen-bonding interaction with Tyr118. The above results show the introduction of isoxazole methoxyl moieties bearing medium side chains plays a significant role in retaining the antifungal activities of target compounds. In addition, some representative compounds exhibited low inhibition profiles for CYP3A4, indicating these new triazole analogues have good safety properties. Further optimization work in our group is in progress.

**Acknowledgments**

This work was supported by the National Natural Science Foundation of China (No. 81673280; 81830106) and National Mega-project for Innovative Drugs (No. 2019ZX09721001).

**Supplementary information**

Supplementary information associated with this article can be found in the online version.

**Reference**

1. Brown, G. D.; Denning, D. W.; Gow, N. A. R.; Levitz, S. M.; Netea, M. G.; White, T. C., Hidden Killers: Human Fungal Infections. *Science Transl Med* **2012,** *4* (165), 165rv13.

2. Brown, G. D.; Denning, D. W.; Levitz, S. M., Tackling human fungal infections. *Science* **2012,** *336* (6082), 647.

3. Mourad, A.; Perfect, J. R., Tolerability profile of the current antifungal armoury. *J Antimicrob Chemother* **2018,** *73* (suppl_1), i26-i32.

4. Perfect, J. R., The antifungal pipeline: a reality check. *Nat Rev Drug Discov* **2017,** *16* (9), 603-616.

5. Choi, J. Y.; Roush, W. R., Structure Based Design of CYP51 Inhibitors. *Curr.Top.Med.Chem.* **2017,** *17*, 30-39.

6. Chai, X.; Zhang, J.; Cao, Y.; Zou, Y.; Wu, Q.; Zhang, D.; Jiang, Y.; Sun, Q., Design, synthesis and molecular docking studies of novel triazole as antifungal agent. *Eur J Med Chem* **2011,** *46* (7), 3167-76.

7. Ni, T.; Pang, L.; Cai, Z.; Xie, F.; Ding, Z.; Hao, Y.; Li, R.; Yu, S.; Chai, X.; Wang, T.; Jin, Y.; Zhang, D.; Jiang, Y., Design, synthesis, and in vitro antifungal evaluation of novel triazole derivatives bearing alkynyl side chains. *Journal of Saudi Chemical Society* **2019,** *23* (5), 576-585.

8. Wu, J.; Ni, T.; Chai, X.; Wang, T.; Wang, H.; Chen, J.; Jin, Y.; Zhang, D.; Yu, S.; Jiang, Y., Molecular docking, design, synthesis and antifungal activity study of novel triazole derivatives. *Eur J Med Chem* **2018,** *143*, 1840-1846.

9. Xie, F.; Ni, T.; Zhao, J.; Pang, L.; Li, R.; Cai, Z.; Ding, Z.; Wang, T.; Yu, S.; Jin, Y.; Zhang, D.; Jiang, Y., Design, synthesis, and *in vitro* evaluation of novel antifungal triazoles. *Bioorg Med Chem Lett* **2017,** *27* (10), 2171-2173.

10. Ritchie, T. J.; Macdonald, S. J. F.; Peace, S.; Pickett, S. D.; Luscombe, C. N., The developability of heteroaromatic and heteroaliphatic rings – do some have a better pedigree as potential drug molecules than others? *MedChemComm* **2012,** *3* (9).

11. Sysak, A.; Obminska-Mrukowicz, B., Isoxazole ring as a useful scaffold in a search for new therapeutic agents. *Eur J Med Chem* **2017,** *137*, 292-309.

12. Zhu, J.; Mo, J.; Lin, H. Z.; Chen, Y.; Sun, H. P., The recent progress of isoxazole in medicinal chemistry. *Bioorg Med Chem* **2018,** *26* (12), 3065-3075.

13. Pesti, J.; Chen, C. K.; Spangler, L.; DelMonte, A. J.; Benoit, S.; Berglund, D.; Bien, J.; Brodfuehrer, P.; Chan, Y.; Corbett, E.; Costello, C.; DeMena, P.; Discordia, R. P.; Doubleday, W.; Gao, Z.; Gingras, S.; Grosso, J.; Haas, O.; Kacsur, D.; Lai, C.; Leung, S.; Miller, M.; Muslehiddinoglu, J.; Nguyen, N.; Qiu, J.; Olzog, M.; Reiff, E.; Thoraval, D.; Totleben, M.; Vanyo, D.; Vemishetti, P.; Wasylak, J.; Wei, C., The Process Development of Ravuconazole: An Efficient Multikilogram Scale Preparation of an Antifungal Agent. *Organic Process Research & Development* **2009,** *13*, 716–728.

14. NCCLS, reference method for broth dilution antifungal susceptibility testing of yeasts; approved standard-second edition, M27-A2, National Committee for Clinical Laboratory Standards, Villanova, PA, **2002**.

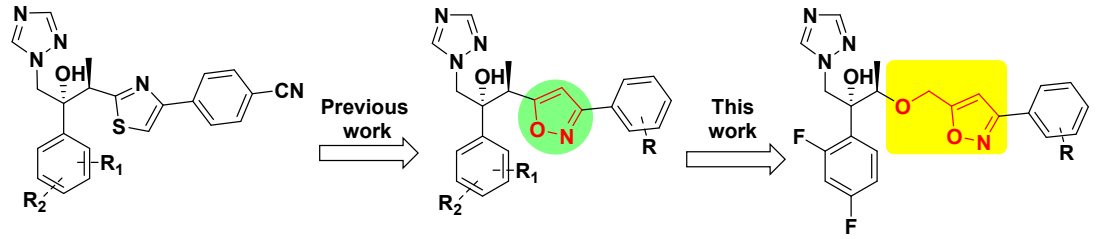
15. Lazzarini, C.; Haranahalli, K.; Rieger, R.; Ananthula, H. K.; Desai, P. B.; Ashbaugh, A.; Linke, M. J.; Cushion, M. T.; Ruzsicska, B.; Haley, J.; Ojima, I.; Del Poeta, M., Acylhydrazones as Antifungal Agents Targeting the Synthesis of Fungal Sphingolipids. *Antimicrob Agents Chemother* **2018,** *62* (5).

**Declaration of interests**

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

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

**Graphical Abstract**




Twenty-six novel triazole analogues featuring isoxazole moieties were designed and synthesized by replacing 4-cyanophenylthioazole moiety in ravuconazole with substituted phenylisoxazole methoxyl. The *in vitro* antifungal activities demonstrate the introduction of isoxazole methoxyl moiety bearing medium side chains is beneficial for retaining the novel compounds antifungal activities.

**Research Highlights**

- Novel triazole analogues featuring isoxazole moieties were designed and synthesized.
- All compounds showed potent activity against *Candida* spp. (MICs ≤0.5 μg/mL).
- The single-crystal data of compound **2** was obtained.
- Compound **a6** showed excellent inhibitory activity against *Candida albicans* and *Candida parasilosis* with $MIC_{80}$ values of 0.0313μg/mL.