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New azoles with antifungal activity: Design, synthesis, and molecular docking

Xiaoyun Chai [a], Jun Zhang [c], Yongbing Cao [b], Yan Zou [a], Qiuye Wu [a,*], Dazhi Zhang [a], Yuanying Jiang [b], Qingyan Sun [a,*]

[a] Department of Organic Chemistry, School of Pharmacy, Second Military Medical University, Guohe Road 325, Shanghai 200433, People's Republic of China
[b] Drug Research Center, School of Pharmacy, Second Military Medical University, Guohe Road 325, Shanghai 200433, People's Republic of China
[c] Overseas Education Faculty of the Second Military Medical University, Xiangyin Road 800, Shanghai 200433, People's Republic of China

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ABSTRACT

In order to search for many target compounds with excellent activities, a series of 1-(1*H*-1,2,4-triazol-1-yl)-2-(2,4-difluoro-phenyl)-3-[(4-substituted phenyl)-piperazin-1-yl]-propan-2-ols were designed, synthesized, and evaluated as antifungal agents. Results of preliminary antifungal tests against eight human pathogenic fungi in vitro showed that all the title compounds exhibited excellent activities with broad spectrum. Moreover, a molecular model for the binding between **5a** and the active site of CACYP51 was provided based on the computational docking results.

Fungal infections pose a continuous and serious threat to human health and life especially to immunocompromised patients.[1–3] Many fungal infections are caused by opportunistic pathogens that may be endogenous (*Candida* infections) or acquired from the environment (*Cryptococcus, Aspergillus* infections). However, besides these known fungal species, new emerging fungal pathogens appear every year as the cause of morbidity and life-threatening infections in the immunocompromised hosts.[1,4]

Nowadays, numerous antifungal drugs with various structures and scaffolds spring up.[5] However, their clinical uses have been limited by the emergence of drug resistance, high risk of toxicity, insufficiencies in their antifungal activity and undesirable side effects. Hence, there is still a need to develop and extend the safe and efficient chemotherapeutic agents with potent antifungal activities.[6]

One of the most common classes of antifungal agents is azoles. For over a decade, azoles have been a mainstay of the antifungal armamentarium. Azoles inhibit the synthesis of ergosterol, the bulk sterol in fungal membranes, by binding to the heme cofactor located in the active site of the cytochrome P450 14α-demethylase (CYP51).[7] Unfortunately, the broad use of azoles has led to development of severe resistance, which significantly reduced their efficacy.[8,9] So the discovery of novel and potent antifungal azoles is the best way to overcome resistance and develop effective therapies.

Researches indicated that the structurally and functionally important regions, such as the heme group, the hydrophilic H-bonding region, the substrate access channel, and the active site have been recognized accurately. The binding mode of azoles with CACYP51 has been investigated by flexible molecular docking.[10–12] The molecular modeling, which gives the utilization of structural information of fungal CYP51s can accelerate the discovery of novel antifungal agents. In our letter, we used the strategy of structure-based rational drug design and find a series of new azoles with excellent in vitro antifungal activity and broad antifungal spectrum.

The general synthetic methodology for the preparation of title compounds 1-(1*H*-1,2,4-triazol-1-yl)-2-(2,4-difluoro-phenyl)-3-[(4-substitutedphenyl)-piperazin-1-yl]-propan-2-ols (**5a–q, 6a–q**) is outlined in Scheme 1. As a key intermediate of our designed triazole antifungals, the oxirane compound **1** was synthesized by the reported procedure.[13] And compound **2** were synthesized according to the literature.[14] The title compound **3** was synthesized by ring-open reaction of oxirane **1** with compound **2**. The good yield was obtained when the reaction was performed in a protic solvent ethanol in the presence of triethylamine as a base at 80 °C. Then the nitro group on the phenyl ring of compound **3** was reduced to an amino group in the presence of Ranney Ni and hydrazine hydrate. In the presence of DMAP (4-dimethylaminopyridine) and EDCI (1-ethyl-3-(3-dimethylaminopropyl) carbodii-mide HCl) in dichloromethane at room temperature, the aniline **4** was converted to title compounds by reacting with various substituted cinnamic acids. All the new compounds (**5a–q, 6a–q**) described above were characterized by IR, ESI, and NMR spectroscopic analysis.[15]

* Corresponding authors. Tel.: +86 21 81871228 15; fax: +86 21 81871225 (Q.S.).
E-mail addresses: wuqy6439@sohu.com (Q. Wu), sqy_2000@163.com (Q. Sun).

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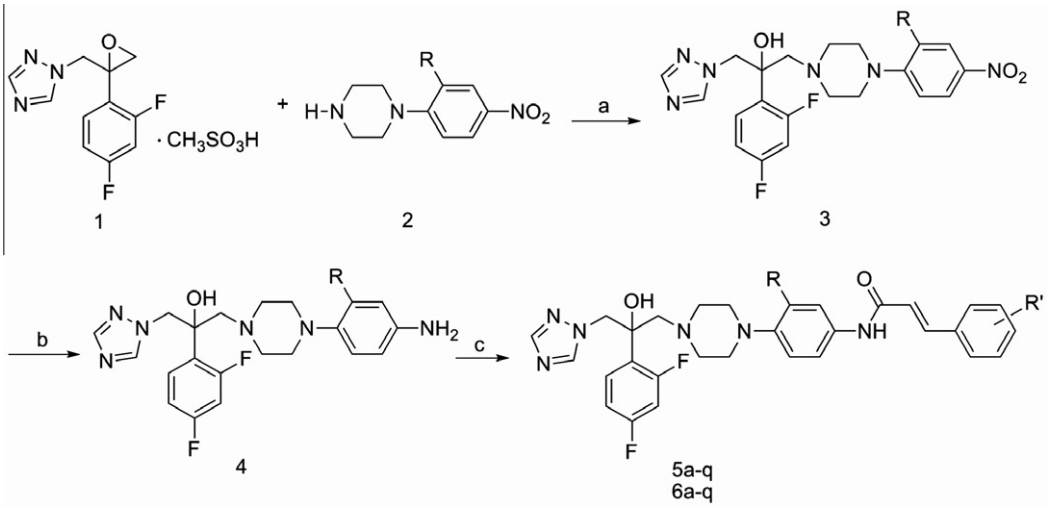

Scheme 1. Conditions: (a) CH_3CH_2OH, Et_3N, 80 °C, 5 h; (b) Ranney Ni, $NH_2NH_2{\cdot}H_2O$, CH_3CH_2OH, 80 °C, 3.5 h; (c) substituted cinnamic acids, DMAP, EDCI, CH_2Cl_2, 8 h.

The in vitro minimal inhibitory concentrations (MICs) of the compounds were determined by the micro-broth dilution method in 96-well microtestplates according to the methods defined by the National Committee for Clinical Laboratory Standards (NCCLS).[16] The 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, the title compounds to be tested were dissolved in dimethyl sulfoxide (DMSO), serially diluted in growth medium, inoculated and incubated at 35 °C. Growth MIC was determined at 24 h for *Candida albicans* and at 72 h for *Cryptococcus neoformans*. Fluconazole (FLC), itraconazole (ICZ), and voriconazole (VCZ) served as the positive control were obtained from their respective manufacturers. The results of assays are summarized in Table 1. The data points from the mean of replicates. All of our susceptibility tests were performed three times by each antifungal agent.

Table 1
Antifungal activities of the title compounds in vitro (MIC_{80}, μg/mL)

Compound	R	R′	*C. albicans*	*C. parapsilosis*	*C. tropicalis*	*C. neoformans*	*T. rubrum*	*F. com.*	*M. gypseum*	*A. fumigatus*
5a	H	H	0.0156	0.0625	0.0625	0.25	0.0039	0.0625	0.25	16
5b	H	2-F	0.0039	0.0625	0.0156	0.0625	0.25	1	0.0625	>64
5c	H	3-F	0.0156	0.25	0.25	0.25	0.25	1	0.0156	4
5d	H	4-F	0.0625	0.25	0.25	0.25	0.0039	1	0.0625	0.0625
5e	H	2-Cl	0.0039	0.25	0.0625	0.25	0.0156	1	0.0156	4
5f	H	3-Cl	0.0625	1	0.25	1	1	64	0.0156	64
5g	H	4-Cl	0.0625	0.25	0.25	1	0.25	4	0.25	64
5h	H	3,4-Cl	0.0156	0.0625	0.0625	0.25	0.0156	0.25	0.25	4
5i	H	3-Br	0.0625	0.25	0.25	1	0.0625	1	1	16
5j	H	4-Br	0.0625	0.25	0.25	0.25	0.0156	1	0.25	4
5k	H	4-CH_3	0.0625	0.25	0.25	1	0.0156	16	0.25	64
5l	H	3,4-OCH_3	0.0156	0.0625	0.0625	0.25	0.25	1	0.25	4
5m	H	2,5-OCH_3	0.0156	0.25	0.0625	0.25	0.25	4	1	>64
5n	H	2-NO_2	0.0156	0.25	0.0625	1	0.0625	4	0.25	>64
5o	H	3-NO_2	0.0156	0.0625	0.25	0.25	0.0625	1	0.25	4
5p	H	4-NO_2	0.0625	1	0.25	1	0.0156	16	0.0625	4
5q	H	3-CN	0.0156	0.25	0.25	1	1	16	0.0625	>64
6a	2-F	H	0.25	0.0625	0.0625	0.0156	0.0625	0.25	0.25	1
6b	2-F	2-F	0.25	0.0625	0.0625	0.25	0.0625	1	0.25	4
6c	2-F	3-F	0.25	0.0156	0.25	0.25	0.25	0.25	0.25	4
6d	2-F	4-F	0.25	0.0156	0.0156	0.25	0.0625	0.25	0.25	1
6e	2-F	2-Cl	1	0.25	0.25	1	0.25	1	0.25	4
6f	2-F	3-Cl	1	0.25	0.0625	0.25	0.25	1	0.0625	1
6g	2-F	4-Cl	0.25	0.0156	0.0625	0.25	0.25	0.25	0.0625	1
6h	2-F	3,4-Cl	1	0.25	0.0625	1	0.0625	0.25	0.25	4
6i	2-F	3-Br	1	0.25	0.0625	0.0156	0.25	1	0.25	4
6j	2-F	4-Br	0.25	0.0625	0.0156	0.0156	0.0625	1	0.0625	1
6k	2-F	4-CH_3	0.25	1	0.0625	4	0.25	1	0.25	1
6l	2-F	3,4-OCH_3	0.25	0.25	1	1	4	4	0.25	>64
6m	2-F	2,5-OCH_3	0.0625	1	0.25	1	0.0625	64	1	>64
6n	2-F	2-NO_2	0.25	1	1	1	0.25	16	1	64
6o	2-F	3-NO_2	0.0039	0.0625	0.0625	0.0625	0.0625	0.0625	0.0625	>64
6p	2-F	4-NO_2	0.0156	0.25	0.25	1	0.0625	1	0.25	>64
6q	2-F	3-CN	0.25	1	1	1	4	64	4	>64
FCZ			4	1	1	1	0.25	16	0.25	64
ICZ			1	0.25	0.25	1	0.0152	0.25	0.0152	1
VCZ			0.0152	1	0.25	0.0152	0.0039	0.0152	0.0039	>64

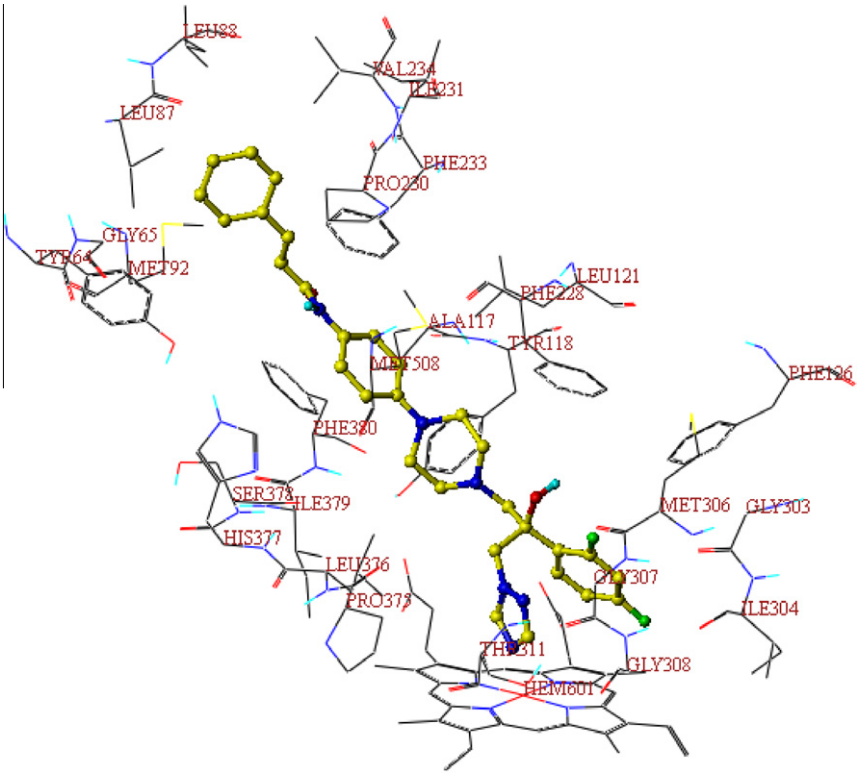

Figure 1. Computed binding geometry of the new inhibitor **5a** in the active site of CYP51.

In vitro antifungal activity assay (Table 1) indicates that all the synthesized compounds (**5a**–**q**, **6a**–**q**) show moderate to excellent activity against nearly all the tested fungal pathogens. The MIC_{80} values indicate that the series of **5a–q** showed more excellent antifungal activities against *C. albicans* than that of the **6a–q** series. Most of the compounds are more excellent than fluconazole and itraconazole with their minimal inhibitory concentration (MIC_{80}) values in the range of 0.0039–1 μg/mL. Noticeably, the MIC value of compounds **5b** and **5e** is 1024 times lower than that of fluconazole against *C. albicans* in vitro and also showed higher activities than that of the other positive controls. *Cryptococcus neoformans* has a worldwide distribution and is the most common cause of life-threatening fungal infections. All the target compounds show higher inhibitory activity against *C. neoformans* than fluconazole and itraconazole with their MIC_{80} values in the range of 0.0156–1 μg/mL. Especially, the inhibitory activity of compounds **6a**, **6i**, and **6j** is 64-fold higher than that of fluconazole and voriconazole. Fluconazole is not effective against *Aspergillus fumigatus*, while our compounds show moderate activity. The MIC_{80} values of compounds **6a, 6d, 6f, 6g, 6j**, and **6k** against *A. fumigatus* are only 1 μg/mL. Moreover, the designed compounds also show good activity against dermatophytes (*Trichophyton rubrum* and *Microsporum gypseum*). For example, compounds **5a** and **5d** (with the MIC_{80} value of 0.0039 μg/mL) are 64 fold more potent against *T. rubrum* than fluconazole.

To clarify the binding mode of our synthesized compounds, compound **5a** was docked into the active site of CACYP51 by the Builder module within InsightII 2000 software package (Fig. 1). The docking results revealed that the compound binds to the active site of CACYP51 through the formation of a coordination bond with iron of heme group. The difluorophenyl group is located in the hydrophobic binding cleft lined with Phe126, Ile304, Met306, Gly307, and Gly308. The long side chain of the compound **5a** is oriented into substrate access channel 2 (FG loop) and forms hydrophobic and van der waals interactions with surrounding hydrophobic residues such as Tyr64, Gly65, Leu87, Leu88, Met92, Ala117, Tyr118, Pro230, Ile231, Phe233, Val234, Leu376, His377, Ser378, Ile379, Phe380, Met508. Furthermore, the phenyl group attached to the piperazinyl of the side chain interacts with the phenol group of Phe380 through the formation of π-π face-to-edge interaction.

In addition, all of the side chains were of the pharmacophores, and the spatial orientations of the pharmacophores were just oriented in the hydrophobic pocket. The side chains of inhibitors were not the determinants for activity, but were very important. They played a role in adjusting the physico-chemical properties of the whole molecule to avoid some dissatisfying side effects and improve their pharmacokinetic and pharmacodynamic behaviors.

In summary, a highly efficient and versatile synthetic method was developed for the synthesis of new azoles. Antifungal avtivity studies of the synthetic compounds suggest that the piperazinyl side chain greatly enhanced the antifungal activity of these analogs against *Candida* species. This observation was explainable by a molecular model resulting from the computational docking simulation, which showed that **5a** could fit into the hydrophobic pocket of CACYP51. The piperazinyl side chain of the compounds is oriented into substrate access channel 2 (FG loop) and forms hydrophobic and van der waals interactions with surrounding hydrophobic residues. Effort aimed at further optimization, as well as in-depth biological investigations, of the identified lead compounds is continuing in our laboratories, and results will be reported in due course.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.12.006.

References and notes

1. Walsh, T. J.; Groll, A.; Hiemenz, J.; Fleming, R.; Roilides, E.; Anaissie, E. *Clin. Microbiol. Infect.* **2004**, *10*, 48.
2. Georgopapadakou, N.; Tkacz, J. *Trends Microbiol.* **1995**, *3*, 98.
3. Nagiec, M.; Nagiec, E.; Baltisberger, J.; Wells, G.; Lester, R.; Dickson, R. *J. Biol. Chem.* **1997**, *272*, 9809.
4. Pfaller, M. A.; Diekema, D. J. *J. Clin. Microbiol. Rev.* **2007**, *20*, 133.
5. Kleeman, A.; Engel, J.; Kutscher, B.; Reichert, D. *Bioseparation* **1999**, *8*, 336.
6. Johnson, D. S.; Li, J. J. *Art Drug Synth.* **2007**, 71.
7. Vanden, B. H.; Willemsens, G.; Marichal, P. *Crit. Rev. Microbiol.* **1987**, *15*, 57.
8. Casalinuovo, I. A.; Di Francesco, P.; Garaci, E. *Eur. Rev. Med. Pharmacol. Sci.* **2004**, *8*, 69.
9. Hoffman, H. L.; Ernst, E. J.; Klepser, M. E. *Expert Opin. Invest. Drugs* **2000**, *9*, 593.
10. Ji, H.; Zhang, W.; Zhou, Y.; Zhang, M.; Zhu, J.; Song, Y.; Lü, J.; Zhu, J. *J. Med. Chem.* **2000**, *43*, 2493.
11. Sheng, C.; Zhang, W.; Ji, H. *Chin. Chem. Lett.* **2004**, *15*, 404.
12. Sheng, C.; Zhang, W.; Ji, H.; Zhang, M.; Song, Y.; Xu, H.; Zhu, J.; Miao, Z.; Jiang, Q.; Yao, J.; Zhou, Y.; Lü, J. *J. Med. Chem.* **2006**, *49*, 2512.
13. Aoyama, Y.; Yoshida, Y.; Sonoda, Y.; Sato, Y. *J. Biol. Chem.* **1987**, *262*, 1239.
14. Sang, W. K.; Jung, G. L.; Eun, J. L.; Hea-Young, P. C.; Chung, Y. Y.; Dae, Y. L.; Kyoung, R. R.; Kim, E. K. *J. Comb. Chem.* **2004**, *6*, 851.
15. *Representative analytical data for compound* **5a**: (*E*)-*N*-(4-{4-[2-(2,4-difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)-propyl]-piperazin-1-yl}-phenyl)-cinna-mamide. Mp: 196–198 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 6.50–7.74 (12H, m, Ar-H), 7.79, 8.15 (2H, ss, triazole-H), 6.83, 7.52 (2H, ss, C=C–H), 4.51–4.60 (2H, dd, triazole-CH_2–), 2.52–3.04 (8H, m, piperazine-H), 2.75–3.14 (2H, dd, CH_2-piperazine–); IR (KBr): 3404, 3284, 3125, 3057, 2822, 1736, 1676, 1616, 1513, 1234, 1137 cm^{-1}. ESI, calcd for $C_{30}H_{30}F_2N_6O_2$, $[M+H]^+$ 545.24, found 545.56.
16. National Committee for Clinical Laboratory Standards, Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts Approved standard. Document M27-A2, National Committee for Clinical Laboratory Standards, Wayne, PA, 2002.