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Original article

Design and synthesis of novel antifungal triazole derivatives with good activity and water solubility

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ABSTRACT

In order to find novel antifungal agents with good activity and aqueous solubility, a series of SYN-2869 analogues containing a pyridine ring were synthesized and evaluated for their *in vitro* antifungal activity and water solubility. The results indicated that some compounds showed potent activity against six pathogenic fungi. In particular, the analogue **17a** having an isobutyl substitution on the triazolone exhibited significant broad spectrum antifungal activity. In addition, the water solubility of compound **17a** was sufficiently improved over SYN-2869.

1. Introduction

During the past two decades, the incidence of invasive fungal infections has rapidly been increasing in immuno-compromised patients emerging from organ transplants, cancer chemotherapy and the HIV epidemic [1], which has caused significant morbidity and mortality in patients [2]. For the treatment of these infections, orally active triazoles, such as Fluconazole and Itraconazole (Fig. 1) have been widely used in clinic use [3]. However, several factors have limited their practical applications. For example, *aspergillous* have become so common in recent years that many experts even believed that *aspergilus* rather than *Candida* is the major problem [4]. But unfortunately, this organism is intrinsically resistant to Fluconazole [5]. In addition, with the increased use of Fluconazole, the number of strains resistant to Fluconazole has increased significantly [6]. The use of another triazole antifungal compound, Itraconazole, which demonstrated stronger activity against *aspergilus* than Fluconazole does, is also hampered by their poor aqueous solubility and absorption [7]. Therefore, the need for novel antifungal agents that exhibit broad spectrum and good water solubility has become more pressing [8].

In our previous research [9], we replaced one of the phenyl rings in Itraconazole with a pyridine ring to obtain a series of novel Itraconazole analogues. Most of the compounds showed excellent *in vitro* antifungal activity. And, more importantly, several of these compounds had higher water solubility and bioavailability than those of Itraconazole. Here we attempt to use this strategy, which has been widely applied in drug design, to obtain more novel potential antifungal agents.

SYN-2869 (Fig. 1) is a novel orally active antifungal agent, which has potent antifungal activity against *Candida* species [10]. However, its efficacy to treat other fungal infections, particularly *Aspergillus fumigatus* and its water solubility are still far from satisfactory [11]. To overcome these drawbacks, we focused our efforts on a similar approach described above that replaces a benzene ring on the SYN-2869 side chain with a pyridine ring to develop a series of novel SYN-2869 analogues.

2. Experimental

The synthetic route of triazolone intermediates **8a–g** with various side chains was outlined in Scheme 1. Compound **2** was obtained by the reaction of compound **1** and mono-*N*-Boc-piperazine in DMF. Compound **2** was reduced with 10% Pd/C under H_2 atmosphere to afford compound **3**. The reaction of compound **3** with phenyl chloroformate and pyridine afforded compound **4**, which was converted to semicarbazide **5** in high

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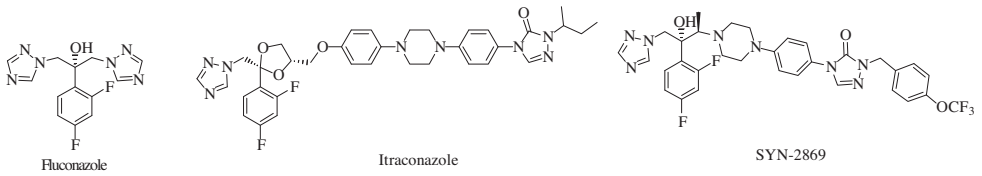
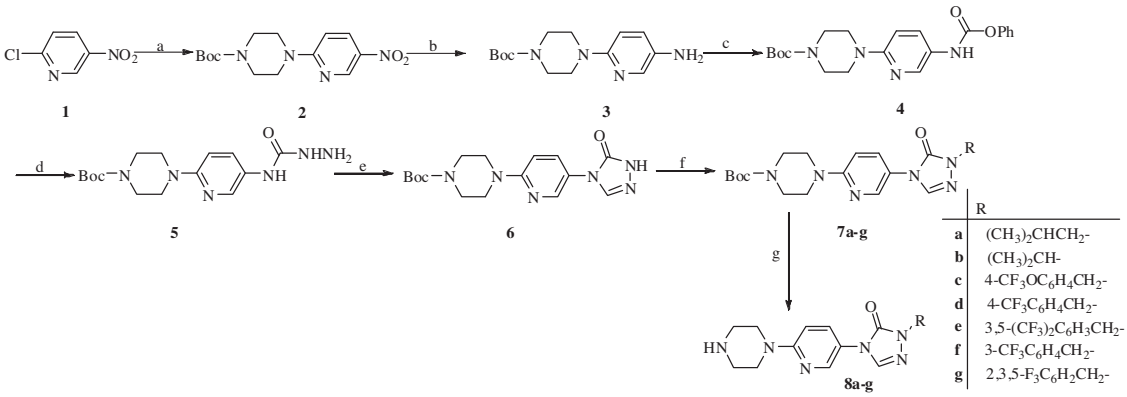


Fig. 1. The structure of Fluconazole, Itraconazole, SYN-2869.



Scheme 1. Conditions and reagents: (a) K_2CO_3, *N*,*N*-dimethyl formamide, *tert*-butylpiperazine-1-carboxylate, 110 °C, 90.0%; (b) H_2, 10% Pd/C, THF, rt, 96.5%; (c) phenyl carbonchloridate, pyridine, CH_2Cl_2, 0 °C, 90.7%; (d) hydrazine hydrate, 1,4-dioxane, reflux, 90.5%; (e) formamidine acetate, DMF, 120 °C, 50.0%; (f) RBr, Cs_2CO_3, DMF, reflux, 50.0–85.0%; (g) trifluoroacetic acid, rt, 80.0–95.0%.

yield. The cyclization of compound **5** afforded the triazolone intermediate **6**. Then, compound **6** was treated with RBr in DMF, giving the substituted triazolone **7a–g**. Finally, triazolone **7a–g** was reacted with trifluoroacetic acid to yield **8a–g**.

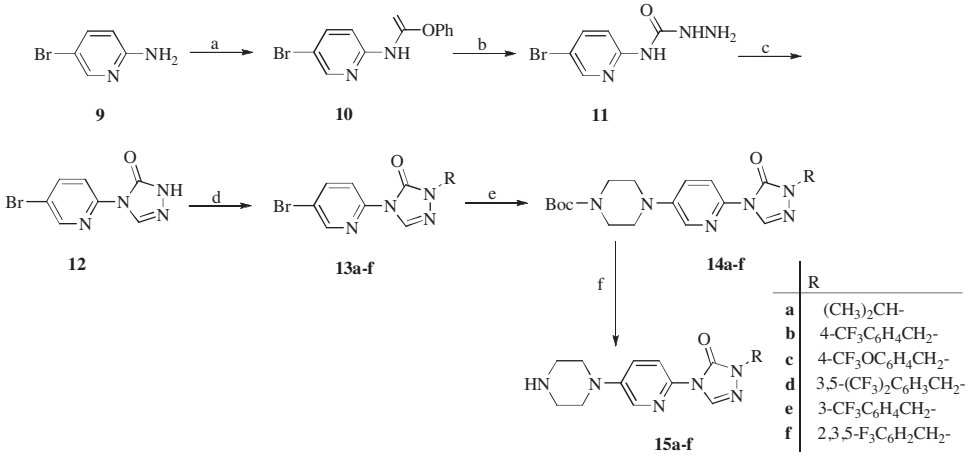
The synthetic route of triazolone **15a–f** was outlined in Scheme 2. The intermediates **13a–f** were obtained *via* similar procedures described in Scheme 1. The coupling reaction between compound **13a–f** and *N*-Boc-piperazine afforded compound **14a–f**, which were mixed and stirred in TFA to yield the key intermediates **15a–f**.

The titled compounds **17a–g** and **18a–f** [12] were synthesized *via* ring-opening reactions between **17a–g** or **18a–f** and (2*R*, 3*S*)-2-(2,4-difluorophenyl)-3-methyl-2-(1*H*-1,2,4-triazole-1-yl)methyl oxirane **16** in the presence of $LiClO_4$ in refluxing acetonitrile (Scheme 3) [13]. The oxirane intermediate **16** was prepared by using a known method [14].

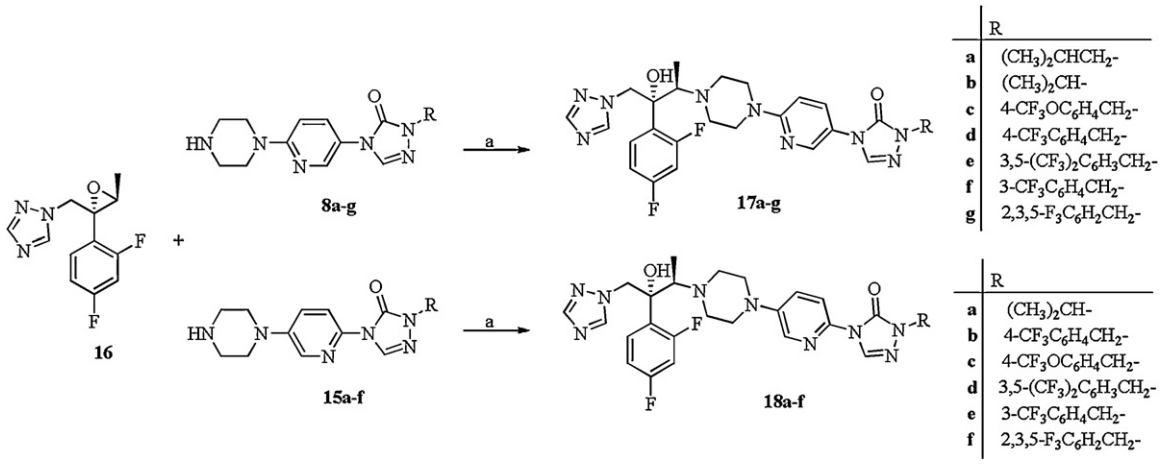
3. Results and discussion

All the titled compounds containing pyridine were new compounds and their structures were confirmed by ^{1}H NMR, ^{13}C NMR, MS-ESI.

Minimum inhibitory concentrations (MICs) of the titled compounds were determined by the method recommended by the National Committee for Clinical Laboratory Standards using the serial dilution method in 96-well microtest plates [15]. Test fungal strains were obtained from ATCC or clinical isolates. The MIC values of all titled compounds were listed in Table 1. All the titled compounds were active against six fungi to some extent. Compared to Fluconazole and Voriconazole, some compounds exhibited stronger or comparable activity against *Candida albicans* and *Cryptococcus neoformans*. The activity of compounds **17a**, **17b**,



Scheme 2. Conditions and reagents: (a) phenyl carbonchloridate, pyridine, CH_2Cl_2, 0 °C, 91.5%; (b) hydrazine hydrate, reflux, 1,4-dioxane, 86.5%; (c) formamidine acetate, DMF, 120 °C, 45.5%; (d) RBr, Cs_2CO_3, DMF, reflux, 55.0–83.6%; (e) *tert*-butylpiperazine-1-carboxylate, $Pd_2(dba)_3$, *t*-BuONa, *rac*-BINAP, toluene, 60 °C, 35.0–65.5%; (f) TFA, rt, 80.0%–95.0%.



Scheme 3. Conditions and reagents: (a) $LiClO_4$, CH_3CN, 80 °C, 41.0–65.0%.

18a, which contained short alkyl side chains, are more potent than control drugs against *Candida parapsilosis*, whereas the compounds with long benzyl side chains showed weaker activity. In addition, compounds **17a** and **18a** also demonstrated much stronger activity against *A. fumigatus* than Fluconazole and SYN-2869. In general, the compounds **17a–g** exhibited higher activity than compounds **18a–f**, which suggested that the position of the N atom in the pyridine ring influenced the antifungal activity of these SYN-2869 analogues.

Meanwhile, we evaluated the water solubility of compounds **17a**, **17b**, 1**7c**, **18a**, **18c** at pH 1.2 and pH 7.4, respectively. As shown in Table 2, all of the tested compounds are more soluble than SYN-2869, especially under acidic conditions, which indicated that the incorporation of a pyridine ring may have been the reason for this improved water solubility. In particular, the short alkyl-substituted compounds **17a**, **17b** and **18a** are more soluble than compounds **17c** and **18c**, which contained a longer benzyl side chain. The combined results indicated that their absorption might be improved.

Table 1
Structures and *in vitro* antifungal activity of title compounds.

Compound	MIC_{80} (μg/mL)					
	C. alb Y0109	*C. alb* SC5314	*C. par*	*C. neo*	*C. gla*	*A. fum*
17a	0.03	0.06	0.25	0.25	4	4
17b	0.06	0.06	1	0.5	16	16
17c	0.125	8	8	0.25	8	16
17d	0.25	8	8	0.25	8	16
17e	16	16	8	8	16	32
17f	4	4	8	4	16	32
17g	8	8	8	16	8	16
18a	0.25	8	0.5	0.5	1	4
18b	4	8	8	2	8	16
18c	8	8	4	8	8	32
18d	8	16	16	8	16	32
18e	4	4	4	4	8	16
18f	8	16	16	16	8	16
SYN-2869	0.06	0.06	0.5	0.25	16	32
FLU	0.5	1	1	2	16	>64
VCZ	0.25	0.5	0.5	0.25	0.125	4

Abbreviations: *C. alb* Y0109, *Candida albicans* Y0109; *C. alb* SC5314, *Candida albicans* SC5314; *C. par*, *Candida parapsilosis*; *C. neo*, *Cryptococcus neoformans*; *C. gla*, *Candida glabrata*; *A. fum*, *Aspergillus fumigatus*; FLU, Fluconazole; VCZ, Voriconazole.

Table 2
Water solubility at different pH value.

Compound	Solubility in water (mg/mL)	
	pH 1.2	pH 7.4
17a	2.86	0.10
17b	3.00	0.04
17c	0.31	0.0039
18a	0.50	0.0056
18c	0.12	0.0021
SYN-2869	BDL	BDL

BDL, below the detection limit.

4. Conclusion

In conclusion, a series of SYN-2869 derivatives containing a pyridine ring were synthesized and evaluated. The results indicated that some titled compounds retained the good activity of SYN-2869. In particular, compound **17a** exhibited the most potent antifungal activity, broadest antifungal spectrum and highest water solubility and is worth further investigations.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.cclet.2013.01.047.

References

[1] (a) L.A. Sorbera, J. Aravamudan, E. Rosa, Therapeutic target for candidiasis, Drugs Future 36 (2011) 627–630;
(b) L.H. Zhu, D.D. Li, Y. Wang, Y.Y. Jiang, Novel thoughts on treatment of invasive fungal infections, Dier Junyi Daxue Xuebao 33 (2012) 558–561 (in Chinese).
[2] (a) A. Jain, S. Jain, S. Rawat, Emerging fungal infections among children: a review on its clinical manifestations, diagnosis, and prevention, J. Pharm. Bioallied Sci. 2 (2010) 314–320;
(b) S. Tobudic, C. Kratzer, E. Presterl, Azole-resistant *Candida* spp. – emerging pathogens? Mycoses 55 (2012) 24–32.
[3] T. Naito, K. Hata, A. Tsuruoka, ER-30346. Triazole antifungal, Drugs Future 21 (1996) 20–24.
[4] I. Hadrich, F. Makni, S. Neji, et al., Invasive aspergillosis: resistance to antifungal drugs, Mycopathologia 174 (2012) 131–141.
[5] K. Wan, Y. Zhang, C. Zhou, Recent advances in antifungal fluconazole, Zhongguo Kangshengsu Zazhi 37 (8–15) (2012) 20 (in Chinese).
[6] J.C.R. Corrêa, H.R.N. Salgado, Review of fluconazole properties and analytical methods for its determination, Crit. Rev. Anal. Chem. 41 (2011) 124–132.

[7] Z.G. Dan, J. Zhang, S.C. Yu, Design and synthesis of novel triazole antifungal derivatives based on the active site of fungal lanosterol 14a-demethylase (CYP51), Chin. Chem. Lett. 20 (2009) 935–938.
[8] G. Kofla, M. Ruhnke, Voriconazole: review of a broad spectrum triazole antifungal agent, Expert Opin. Pharmacother. 6 (2005) 1215–1229.
[9] Y. Liu, Z.N. Liu, X.F. Cao, Design and synthesis of pyridine-substituted itraconazole analogues with improved antifungal activities, water solubility and bioavailability, Bioorg. Med. Chem. Lett. 21 (2011) 4779–4783.
[10] R.A. Fromtling, J. Castaner, Syn-2869: antifungal, Drugs Future 24 (1999) 30–37.
[11] E.M. Johnson, A. Szekely, D.W. Warnock, In vitro activity of Syn-2869, a novel triazole agent, against emerging and less common mold pathogens, Antimicrob. Agents Chemother. 43 (1999) 1260–1263.
[12] Selected data of the titled compounds. **17a**: White solid, yield 31.1%, mp 179–180 °C; $[\alpha]_D^{22}$ −59.2 (*c* 0.25, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.22 (d, 1H, *J* = 2.74 Hz), 7.88 (s, 1H), 7.76 (s, 1H), 7.69 (dd, 1H, *J* = 2.75, 9.15 Hz), 7.56 (s, 1H), 7.48–7.42 (m, 1H), 6.81–6.72 (m, 3H), 5.05 (s, 1H), 5.01–4.79 (m, 2H), 3.72–3.45 (m, 6H), 3.11–3.01 (m, 3H), 2.61–2.43 (m, 2H), 2.11–2.06 (m, 1H), 0.98 (d, 3H, *J* = 7.02 Hz), 0.96 (d, 6H, *J* = 6.68 Hz); ^{13}C NMR (101 MHz, $CDCl_3$): δ 158.42, 152.48, 151.65, 144.19, 142.27, 133.64, 132.81, 130.70 (dd, *J* = 9.0, 6.4 Hz), 124.73, 124.66, 121.08, 111.63, 111.45, 106.83, 104.09, 78.79, 64.20, 55.93, 55.87, 52.89, 45.72, 28.26, 19.86, 6.98; MS (ESI) *m*/*z* (554.8 [M+1]). **18b**: White solid, yield 41.5%, mp 78–79 °C; $[\alpha]_D^{22}$ −57.2 (*c* 0.25, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.35 (s, 1H), 8.17 (d, 1H, *J* = 8.84 Hz), 8.11 (d, 1H, *J* = 3.05 Hz), 7.87 (s, 1H), 7.79 (s, 1H), 7.63 (d, 2H, *J* = 8.54 Hz), 7.53–7.38 (m, 3H), 7.37 (dd, 1H, *J* = 3.05 Hz, *J* = 8.84 Hz), 6.81–6.65 (m, 2H), 5.05 (s, 1H), 4.96–4.79 (m, 2H), 3.32–3.18 (m, 4H), 3.15–3.01 (m, 3H), 2.65–2.46 (m, 2H), 0.97 (d, 3H, *J* = 5.8 Hz); ^{13}C NMR (101 MHz, $CDCl_3$): δ 151.72, 151.33, 145.95, 144.19, 140.00, 139.40, 135.76, 133.27, 130.68, 130.41, 130.03, 128.50, 125.73, 125.12, 124.76, 124.62, 122.63, 78.93, 64.03, 55.95, 55.88, 49.12, 48.68, 6.93; MS (ESI) *m*/*z* (656.2 [M+1]).
[13] H. Ogura, H. Kobayashi, K. Nagai, Synthesis and antifungal activities of (2R, 3R)-2-aryl-1-azolyl-3-(substituted amino)-2-butanol derivatives as topical antifungal agents, Chem. Pharm. Bull. 47 (1999) 1417–1425.
[14] J. Pesti, C.K. Chen, L. Spangler, The process development of ravuconazole: an efficient multikilogram scale preparation of an antifungal agent (1), Org. Process. Res. Dev. 13 (2009) 716–728.
[15] Clinical and Laboratory Standards Institute/National Committee for Clinical Laboratory Standards: Reference method for broth dilution antifungal susceptibility testing of Yeast, Approved Standard, 3rd ed., Document M27-A3. Wayne, PA, Clinical and Laboratory Standards Institute, 2009.