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Bioorganic & Medicinal Chemistry Letters 18 (2008) 3261–3265
Bioorganic & Medicinal Chemistry Letters

Synthesis and SAR studies of biaryloxy-substituted triazoles as antifungal agents

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Received 7 December 2007; revised 30 March 2008; accepted 21 April 2008
Available online 25 April 2008

Abstract—A series of 1-(substituted biaryloxy)-2-(2,4-difluorophenyl)-3-(1*H*-1,2,4-triazol-1-yl) propan-2-ol were synthesized and their antifungal activities were evaluated against eight human pathogenic fungi in vitro. Seventeen compounds showed activity 4- to 64-fold higher than voriconazole against *Candida albicans*. SAR clearly suggested that introduction of a biaryloxy side chain greatly enhanced the antifungal activity of triazole analogs against *Candida* species.
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Systemic fungal infections are life-threatening and have become increasingly common in the immunocompromised hosts.[1] Currently, triazole drugs (fluconazole, itraconazole, voriconazole, and posaconazole) are the most frequently used antifungals in clinic.[2] However, resistance to azoles is emerging and may pose a serious health problem in the future.[3] In addition, triazole drugs are often associated with hepatotoxicity and limited antifungal spectrum.[4,5] Consequently, it remains attractive to develop new triazole derivatives possessing broader antifungal spectra and higher therapeutic indexes.

In the past three decades, SAR of antifungal triazoles has been extensively studied.[6,7] These studies have revealed a pharmacophore, bolded in Figure 1, which contains a triazole ring linked to a dihalophenyl ring through a two carbon chain. In addition, the carbon alpha to the phenyl ring bears a hydroxyl group. The pharmacophore is critical for the binding of triazoles with the active site of CYP51 (lanosterol 14-demethylase), the enzyme which is required for the biosynthesis of ergosterol, an essential component of fungal cell membrane.[8] Therefore, current researches are mainly focused on the optimization of the side chain attached to the pharmacophore. Optimization of the side chain has led to new compounds with better biological and/or pharmacological properties.[6,9,10]

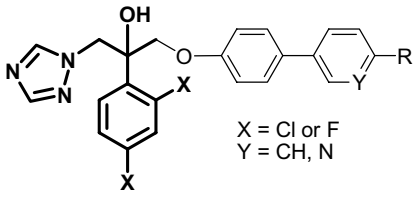

Figure 1. Structure of biaryloxy triazole derivatives.

The side chains in many triazole antifungals bear one or two 1,4-disubstituted aryl substituents for improved antifungal activity.[6,7] However, the lipophilicity of these aryl substitutents, in addition to the hydrophobic dihalophenyl group in the triazole pharmacophore, could give rise to poor water solubility of the antifungals to prevent their clinical applications. Herein, we wish to report the synthesis and SAR of a series of triazole derivatives bearing a variety of biaryloxy side chains. These compounds were designed by introducing a variety of

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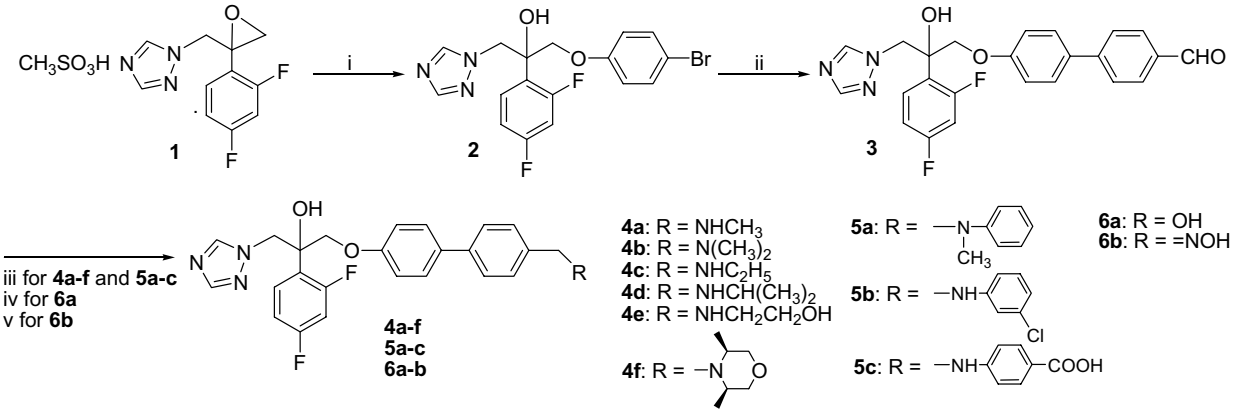
doi:10.1016/j.bmcl.2008.04.056

hydrophilic substituents into the biphenyloxy or 4-(pyridin-3-yl)phenyloxy side chain to improve their solubility and antifungal activity.

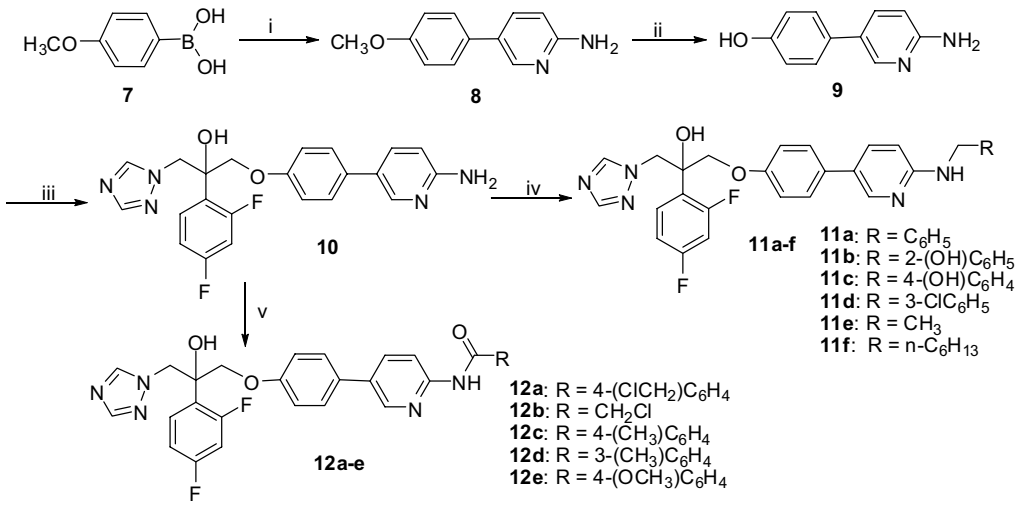
Synthesis of the biphenyloxy derivatives **4**–**14** from oxirane **1** was accomplished using chemistry illustrated in Scheme 1. Oxirane **1** was allowed to react with 4-bromophenol in the presence of K_2CO_3 in DMF to provide compound **2**. Suzuki coupling of **2** with 4-formylphenylboronic acid afforded the aldehyde intermediate **3**.[11,12] The target compounds **4a**–**f** and **5a**–**c** were obtained by reductive amination of aldehyde **3** with the corresponding amines by $NaBH_3CN$ in methanol.[13] Compounds **6a** and **6b** were obtained by the $NaBH_4$ reduction or oximination of the aldehyde **3**, respectively.

Synthesis of the 4-(pyridine-3-yl)phenyloxy derivatives **11a**–**f** and **12a**–**e** is outlined in Scheme 2. Suzuki coupling of 4-methoxyphenylboronic acid (**7**) with 5-bromopyridin-2-amine afforded the biaryl intermediate **8**. The methyl group of **8** was removed by refluxing **8** with HBr. Reaction of **9** with compound **1** in the presence of K_2CO_3 in DMF afforded amine **10.** The target compounds **11a**–**f** were obtained by reductive amination of **10** with the corresponding aldehydes by $NaBH_3CN$. Compounds **12a**–**e** were prepared by reacting **10** with the corresponding acyl chlorides in pyridine.[14] Compounds **13a**–**f** were prepared by reacting the chloride intermediate **12a** or **12b** with the corresponding amines in acetonitrile, respectively (Scheme 3). All the new compounds (**3**–**6**, **10**–**14**) described above were characterized by IR, HRMS, and NMR spectroscopic analysis.[15]

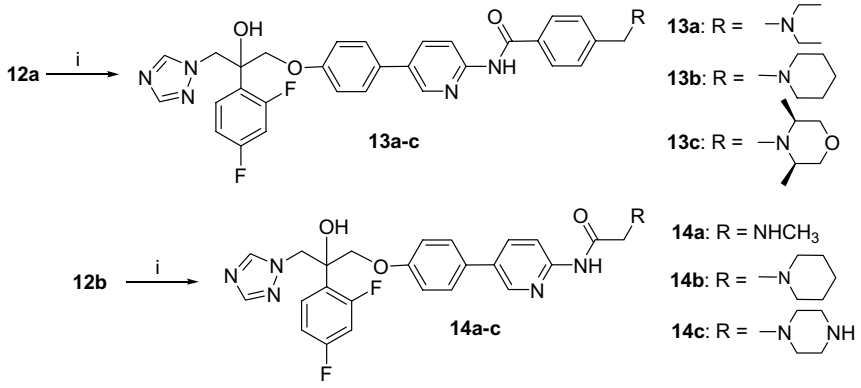
The in vitro antifungal activities of the 30 novel biaryloxy-substituted triazole derivatives were evaluated by the standard broth microdilution method of the NCCLS.[16] The tested fungi species included four yeast species (*Candida albicans, Candida tropicalis*, *Candida parapsilosis*, and *Cryptococcus neoformans*) and four mold species (*Aspergillus fumigatus*, *Trichophyton rubrum*, *Microsporum gypseum*, and *Fonsecaea compacta*). The positive controls included four different classes of



Scheme 1. Synthesis of the biphenyloxy derivatives **4a**–**f**, **5a**–**c**, and **6a**–**b**. Reagents and conditions: (i) 4-bromophenol, K_2CO_3, DMF, 90 °C, 65%; (ii) 4-formylphenylboronic acid, $Pd(OAc)_2$, PPh_3, Na_2CO_3, 2-propanol, reflux, 57%; (iii) RH, $NaBH_3CN$, MeOH, 33–56%; (iv) 10%Pd/C, H_2, MeOH, 85%; (v) $NH_2OH{\cdot}HCl$, MeOH, 97%.



Scheme 2. Synthesis of the 4-(pyridine-3-yl)phenyloxy derivatives **11a**–**f** and **12a**–**e**. Reagents and conditions: (i) 5-bromopyridin-2-amine, $Pd(OAc)_2$, PPh_3, Na_2CO_3 (2 N), 2-propanol, reflux, 63%; (ii) HBr, reflux, 98%; (iii) compound **1**, K_2CO_3, DMF, 90 °C, 47%; (iv) RCHO, $NaBH_3CN$, MeOH, 37–70%; (v) RCOCl, Pyr, 0 °C, 42–64%.



Scheme 3. Synthesis of the 4-(pyridine-3-yl)phenyloxy derivatives **13a–c** and **14a–c**. Reagents and conditions: (i) RH, CH_3CN, reflux, 55–81%.

Table 1. In vitro antifungal activity of biaryloxy triazole derivatives

Compound	MIC_{80}[a] (μg/mL)							
	Yeasts[b]				Molds[b]			
	C. albicans	*C. parapsilosis*	*C. tropicalis*	*C. neoformans*	*A. fumigatus*	*T. rubrum*	*M. gypseum*	*F. compacta*
3	0.0625	>64	0.25	1	>64	1	0.25	0.25
4a	0.25	0.25	0.25	64	>64	0.0625	1	4
4b	0.25	0.25	0.0625	4	64	0.0625	1	4
4c	1	0.25	0.25	16	>64	0.25	1	1
4d	1	0.0625	0.0625	16	>64	0.25	1	4
4e	4	1	1	64	>64	1	16	16
4f	0.0625	0.0625	0.0625	0.25	>64	0.25	4	4
5a	1	1	0.25	1	>64	16	>64	16
5b	0.25	0.25	0.25	>64	>64	1	>64	>64
5c	0.0156	0.0625	0.0039	>64	16	0.25	1	1
6a	0.0156	0.0625	0.0156	1	>64	1	1	16
6b	0.0039	0.0039	0.0156	1	>64	1	1	1
10	0.0625	0.25	0.25	64	4	0.0156	0.25	0.0625
11a	0.25	0.0625	0.25	1	4	0.25	>64	4
11b	0.0625	0.0625	0.0625	>64	64	0.0625	0.0625	0.25
11c	0.0625	0.0625	0.0156	>64	>64	>64	4	1
11d	4	0.25	0.25	4	>64	4	1	4
11e	0.0625	0.0625	0.0625	16	>64	>64	>64	1
11f	0.25	1	0.25	1	>64	0.25	1	1
12a	0.0156	0.0625	0.0625	0.25	>64	0.25	1	1
12b	0.25	0.25	0.25	1	>64	0.25	1	1
12c	0.0625	0.0625	0.0625	0.25	>64	0.0625	0.25	1
12d	0.25	0.25	0.25	0.25	64	1	1	1
12e	0.0625	0.25	0.25	1	64	0.25	1	0.25
13a	0.0625	0.25	0.0625	4	>64	1	1	0.25
13b	0.0625	0.0625	0.0625	1	4	0.25	1	1
13c	0.0625	0.0625	0.0625	4	>64	>64	>64	16
14a	0.0625	0.25	0.25	16	4	1	1	1
14b	0.0625	0.0625	0.0156	1	>64	0.25	0.25	1
14c	16	4	1	64	>64	1	1	1
FCZ[c]	16	4	1	0.25	>64	1	1	16
VCZ[c]	0.25	0.0625	0.0156	1	0.25	0.0156	0.0156	0.0625
ICZ[c]	0.5	0.0312	0.25	1	1	0.0156	0.0625	0.0625
TBL[c]	1	0.25	0.25	0.25	0.0625	0.0625	0.0156	0.0625
KCZ[c]	0.25	0.0625	0.0625	0.0156	1	0.25	0.25	0.25
AMB[c]	0.25	1	4	1	16	16	1	16

[a] The tested concentration ranges were from 0.00024 to 64 μg/mL. The given data are mean values of three parallel experiments.

[b] *Candida albicans* ATCC 76615, *Cryptococcus neoformans* ATCC 32609. The other tested organisms are clinic isolates obtained from Chinese Changhai Hospital's Fungi Culture Collection.

[c] Fluconazole (FCZ), voriconazole (VCZ), itraconazole(ICZ), terbinafine (TBL), ketoconazole (KCZ), and amphotericin B (AMB) were used as the positive controls.

antifungal drugs currently used in clinic, including triazoles (fluconazole, voriconazole, and itraconazol), allylamine (terbinafine), imidazole (ketoconazole), and polyene (amphotericin B). The minimum 80% inhibitory concentration (MIC_{80}) values are summarized in Table 1.

The MIC_{80} values indicate that nearly all the biaryloxy derivatives showed excellent antifungal activities against the three yeast *Candida* species including *C. albicans*, *C. parapsilosis*, and *C. tropicalis*. Noticeably, more than half (18) of the 30 compounds showed higher activity against *C. albicans* than the tested six conventional drugs. Compared to voriconazole, one of the most prescribed antifungals in clinic, these compounds were 4- to 64-fold more effective (MIC_{80} = 0.0156–0.0625 μg/mL). These results clearly suggested that introduction of a biaryloxy side chain to the triazole pharmacophore greatly enhanced the antifungal activity of these analogs against *Candida* species. The yeast species *C. neoformans* was found to be much less sensitive to these biaryloxy derivatives, as indicated by their MIC_{80} values showing moderate to no activity.

Compared to their strong activities against yeast species, the MIC_{80} values indicate that the activities of these biaryloxy derivatives against mold fungi are much lower. Nearly all the 30 compounds showed no activity against *A. fumigatus* and majority showed only moderate activity against *M. gypseum* and *F. compacta*. However, these compounds showed superior activity against *T. rubrum*, with the lowest MIC_{80} value (0.0156 μg/mL for Compound **10**) same as that of voriconazole and itraconozole. The inactivity of these biaryloxy derivatives against *A. fumigatus* was not a surprise because it has been known that this mold species possesses an intrinsic mechanism resistant to triazole antifungals.[17]

Of the 30 biaryloxy derivatives, only compounds **10** and **11b** are effective against both *Candida* and mold species. Their broad antifungal spectra are comparable to that of voriconazole, itraconozole, and terbinafine, and more than that of ketoconazole and amphotericin B. Interestingly, both compounds are 4-(pyridin-3-yl)phenyloxy derivatives. This result suggests that the antifungal spectrum of the biaryloxy triazole analogs benefits more from 4-(pyridin-3-yl)phenyloxy than the biphenyloxy substituent. The superiority of the former could be attributed to its higher hydrophilicity given by the pyridine ring, subsequently benefiting the in vivo absorption and distribution of the analog.

Among the 12 biphenyloxy derivatives, compounds **4a–e** with aliphatic amino substituents showed mainly moderate antifungal activities (MIC_{80} = 0.25–1 μg/mL) against *C. albicans*. Replacing the amino group with a 2,6-dimethyl-morpholinyl group in compound **4f** gave rise to a 4- to 16-fold increase on the activity. Compounds **5a** and **5b** with aromatic amino substituents showed moderate activity against all three *Candida* species. Introduction of a carboxylic group in para to the amino group in compound **5c** gave rise to a significant 4- to 64-fold increase on the activity. Interestingly, the 2,6-dimethyl-morpholinyl group in **4f** is similar to the 4-hydrocarbonyl-phenyl group in **5c**, both having two polar groups (O and NH) connected through a hydrophobic linker. Given that both compounds have higher antifungal activity than their parallel analogs, respectively, this structural similarity between **4f** and **5c** suggests the presence of a corresponding hydrogen bonding site and a hydrophobic region in the CYP51 substrate binding site.

Replacing the amino groups with a hydroxyl in **6a** or oxime group in **6b** resulted in enhanced activity. The hydroxyl compound **6a** showed not only activity comparable to **5c** against *Candida* but also activity against *C. neoformans* which is absent in the latter. The oxime derivative **6b** was found to be the most effective compound against *Candida* among the tested 30 compounds. Its activity was 64- and 16-fold higher than that of voriconazole against *C. albicans* and *C. parapsilosis*, respectively. The significance of the discovery of **6b** is reinforced by the fact that *C. albicans* is the most frequently encountered *Candida* species and responsible for most serious invasive fungal infections in clinic.[18]

In the 4-(pyridin-3-yl)phenyloxy series, compounds **11a–f** with amino substitutents showed similar activities as compounds **12a–e** with amides. Compared to the non-substituted compound **10**, most of the N-substituted compounds showed slightly higher antifungal activity against yeasts, suggesting that the activity may be benefited from additional substituents. However, compared to compound **10**, nearly all the N-substituted compounds suffered activity loss against mold fungi. Interestingly, compounds (**4f**, **5a–c**) in the biphenyloxy series with bulky substitutents are also the least effective compounds against molds. These results indicated that additional bulky substituents deteriorated the antifungal activity of these triazole analogs against molds. The 4-(chloromethyl)benzamide **12a** was found to be the most effective compound among the 4-(pyridin-3-yl)phenyloxy series against yeast fungi, 4-fold more effective than voriconazole against *C. albicans*. The least effective compound was found to be **14c** which had a piperazine substitutent. Comparison between **13c** and **14c**, **13b–c** suggests that the amino group at the side chain terminal is not favored by the enzyme.

In conclusion, a series of novel biaryloxy-substituted triazole antifungal agents were synthesized and their antifungal activities were screened for both yeast and mold fungi species. These analogs showed improved in vitro antifungal activities especially against *Candida* species. This research has led to the discovery of compounds **5c**, **10**, and **12a** for further optimizations.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (Nos. 30672533 and 30772644). We thank Dr. Yongying Jiang, Dr. G. Karthikeyan, and Professor Tingsen Zhou for their advice and discussions.

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15. *Representative analytical data for* ***4f***: ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.04 (s, 1H), 7.83 (s, 1H), 7.65–7.61 (m, 1H), 7.51–7.48 (m, 4H), 7.36 (d, 2H, J = 7.8 Hz), 6.94–6.92 (m, 2H), 6.88–6.78 (m, 2H), 4.87 (dd, 2H, J = 14.4, 23.4 Hz), 4.60 (s, 1H), 4.29 (q, 2H, J = 9.6 Hz), 3.72–3.69 (m, 2H), 3.50 (s, 2H), 2.73 (d, 2H, J = 10.2 Hz), 1.77 (t, 2H, J = 10.8 Hz), 1.15 (d, 6H, J = 19.8 Hz); IR (cm^{-1}, KBr): 3126, 2972, 2931, 2870, 1616, 1500, 1457, 1422, 1273, 1243, 1141, 1082, 966, 851, 824, 678, 517; HRMS (*m*/*z*): calcd for $C_{30}H_{33}F_2N_4O_3$: 535.2521; found: 535.2616.
Compound ***11a***: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.22 (d, 1H, J = 1.8 Hz), 8.05 (s, 1H), 7.86 (s,1H), 7.48–6.81 (m, 14H), 6.47 (d, 1H, J = 8.7 Hz), 4.88 (d, 2H, J = 4.5 Hz), 4.55 (d, 2H, J = 6.0 Hz), 4.28 (d, 2H, J = 4.2 Hz); IR (KBr, cm^{-1}): 3234, 3121, 3029, 2925, 2849, 1605, 1512, 1421, 1388, 1279, 1245, 1138, 1059, 1028, 968, 814, 701, 678, 548; HRMS (*m*/*z*): calcd for $C_{29}H_{26}F_2N_5O_2$: 514.2055; found: 514.2039.
Compound ***12a***: ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.67 (d, 1H, J = 2.4 Hz), 8.37 (s, 1H), 8.26 (d, 1H, J = 8.7 Hz), 8.11 (dd, 1H, J = 2.7, 8.7 Hz), 8.04 (d, 2H, J = 8.1 Hz), 7.81 (s, 1H), 7.68 (d, 2H, J = 8.7 Hz), 7.59 (d, 2H, J = 8.4 Hz), 7.51 (dd, 1H, J = 7.2, 9.0 Hz), 7.25–7.17 (m, 1H), 7.08–7.02 (m, 3H), 4.85 (s, 2H), 4.75 (d, 2H, J = 4.2 Hz), 4.40 (d, 1H, J = 10.2 Hz), 4.26 (d, 1H, J = 10.5 Hz); IR (KBr, cm^{-1}): 3387, 3130, 2960, 2876, 1674, 1615, 1501, 1422, 1378, 1303, 1272, 1244, 1140, 1056, 967, 828, 711, 679, 523; HRMS (*m*/*z*): calcd for $C_{30}H_{25}ClF_2N_5O_3$: 576.1614; found: 576.1595.
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