Full Paper

# Synthesis and Antifungal Activity of 2-Aryl-1,2,4-triazolo[1,5-a]pyridine Derivatives

**Yun Luo, Yongzhou Hu**

Department of Medicinal Chemistry, School of Pharmaceutical Sciences, Zhejiang University, Hangzhou, China

A series of novel antifungal triazole derivatives 2-aryl-1,2,4-triazolo[1,5-a]pyridine **9a–m** were synthesized and tested *in vitro* for their growth inhibitory activities against *C. albicans* and *T. rubrum*. The MIC values indicate that the activities of three compounds were superior or comparable to fluconazole against both tested fungi, worthy of further investigation of its antifungal activities.

**Keywords:** Synthesis / Antifungal / 2-Aryl-1,2,4-triazolo[1,5-a]pyridine

Received: October 20, 2005; Accepted: December 20, 2005

DOI 10.1002/ardp.200500227

## Introduction

The rising incidence of fungal infections, along with the emergence of fungal resistance to conventionally utilized azole antifungals and severe toxicity of amphotericin B, has added considerable urgency to the pursuit of safe and effective therapies in the past decade [1–3]. In continuation of our current research on the synthesis and biological evaluation of small bioactive heterocyclic molecules [4, 5], a new series of 2-aryl-1,2,4-triazolo[1,5-a]pyridines were synthesized and their potential antifungal activities were evaluated.

### Chemistry

The 2-aryl-1,2,4-triazolo[1,5-a]pyridine structure was synthesized by 1,3-dipolar cycloaddition of substituted *N*-aminopyridinium mesitylenesulfonate with aromatic nitriles in the presence of potassium hydroxide solution. Different heterocycles were introduced to this structure. Thirteen compounds **9a–m** were designed and synthesized for evaluating their possible antifungal effect (Scheme 1). The compounds **9a–m** were characterized by $^{1}$H-NMR, $^{13}$C-NMR, and physical data are given in Table 1.

**Correspondence:** Yongzhou Hu, Department of Medicinal Chemistry, College of Pharmaceutical Sciences, Zhejiang University, Hangzhou 310031, China.
**E-mail**: huyz@zjuem.zju.edu.cn
**Fax**: +86 571 87217412

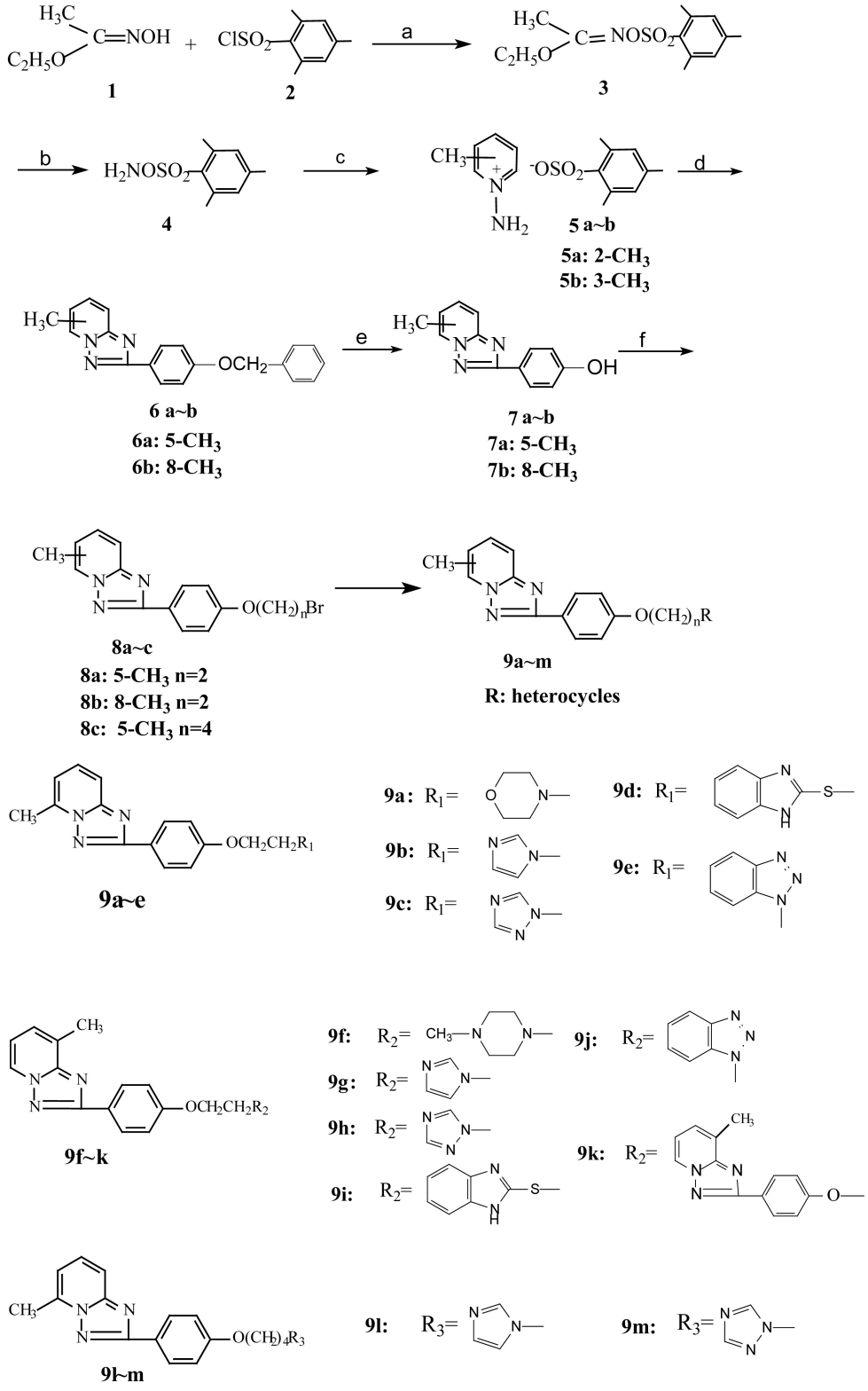
## Results and discussion

The thirteen 2-aryl-1,2,4-triazolo[1,5-a]pyridine compounds **9a–m** were tested *in vitro* for their growth-inhibitory activities against pathogenic fungi by the standard method. The MIC (minimum inhibitory concentration) values against *Candida albicans 7374* and *Trichophyton rubrum 6180* are presented in Table 2. We chose fluconazole and amphotericin B as fungicidal standard agent [6].

As indicated in the Table 2, compounds **9a**, **9f** and **9j** showed the same potent antifungal activities against *C. albicans* ($MIC_{50}$ 32 mg/mL) as fluconazole ($MIC_{50}$ 32 mg/mL). And compounds **9g**, **9k** and **9l** showed also good activities against *C. albicans* ($MIC_{50}$ 64 mg/mL).

The activities of these thirteen compounds against *T. rubrum* were less potent than those against *C. albicans*. However, compound **9a**, **9f** ($MIC_{50}$ 128 mg/mL) were superior to those of fluconazole ($MIC_{50}$ 256 mg/mL) and **9c**, **9e**, **9j**, **9k**, **9l**, **9m** ($MIC_{50}$ 256 mg/mL) were comparable to those of fluconazole against *T. rubrum*.

The number of cases of *in vitro* resistance of fluconazole has risen dramatically. It is interesting to know that from our thirteen new compounds, six **9a, 9f, 9g, 9j, 9k,** and **9l**, have activities against *Candida albicans* ($MIC_{50}$ 32–64 mg/mL), in which **9a**, **9f**, and **9j** were superior or comparable to those of fluconazole against both tested fungi. So far, we have not obtained the structure-activity relationship for this kind of new compounds. Because of lack-




**Scheme 1.** Synthesis and structure of 2-aryl-1,2,4-triazolo[1,5-a]pyridine compounds **9a–m**. *DMF: *N,N*-dimethyl formamide, CTAB: cetyl-trimethylammonium bromide. a: $Et_3N$ / DMF / 0°C / 0.5 h; b: 70% $HClO_4$ / 1,4-dioxane / 0 °C / 20 min; c: 2-methyl pyridine or 3-methyl pyridine / $CH_2Cl_2$ / rt / 1 h; d: 4-benzyloxybenzonitrile / 2N KOH / EtOH / rt / 24 h; e: $H_2$ / 10% Pd-C / DMF / 6 h; f: 1,2-dibromoethane or 1,4-dibromobutane / CTAB / NaOH / $H_2O$ / flush.

**Table 1.** Structure data of the products **9a–m**.

| N°. | m.p. [°C] | Yield [%] | Formula | $^1$H-NMR (400 or 500 MHz, $CDCl_3$ δ ppm) for thirteen compounds $^{13}$C-NMR (100.6 MHz, $CDCl_3$) for **9g** |
|---|---|---|---|---|
| **9a** | 134–136 | 97 | $C_{19}H_{22}N_4O_2$ | 2.62 (brs, 4H, $-CH_2NCH_2-$), 2.83–2.86 (m, 5H, $-NCH_2-$ and 5-$CH_3$), 3.76 (t, J = 4.5 Hz, 4H, $-CH_2OCH_2-$), 4.20 (t, J = 5.6 Hz, 2H, $-CH_2O-$), 6.81 (d, J = 7.0 Hz, 1H, 6-H), 7.02, 8.25 (AA′BB′, each 2H, J = 8.7 Hz, 2 × Ar-2H), 7.42 (t, J = 7.0 Hz, J = 8.8 Hz, 1H, 7-H), 7.61 (d, J = 8.8 Hz, 1H, 8-H) |
| **9b** | 139–141 | 88 | $C_{18}H_{17}N_5O$ | 2.84 (s, 3H, 5-$CH_3$), 4.31 (t, 2H, $-OCH_2CH_2-$), 4.39 (t, 2H, $-OCH_2CH_2-$), 6.81 (d, 1H, 6-H), 8.28, 7.00 (AA′BB′, each 2H, 2 × Ar-2H), 7.11 (d, 1H, imidazole 5′-H), 7.44 (t, 1H, 7-H), 7.61 (d, 1H, 8-H), 7.66 (s, 1H, imidazole 4′-H), 8.20 (s, 1H, imidazole 2′-H) |
| **9c** | 141–143 | 85 | $C_{17}H_{16}N_6O$ | 2.84 (s, 3H, 5-$CH_3$), 4.42 (t, 2H, $-OCH_2CH_2-$), 4.62 (t, 2H, $-OCH_2CH_2-$), 6.83 (d, 1H, 6-H), 8.27, 6.99 (AA′BB′, each 2H, 2 × Ar-2H), 7.43 (t, 1H, 7-H), 7.63 (d, 1H, 8-H), 7.99 (s, 1H, triazole 3′-H), 8.25 (s, 1H, triazole 5′-H) |
| **9d** | 212–214 | 95 | $C_{22}H_{19}N_5OS$ | 2.82 (s, 3H, 5-$CH_3$), 3.68 (t, 2H, $-OCH_2CH_2-$), 4.36 (t, 2H, $-OCH_2CH_2-$), 6.80 (d, 1H, 6-H), 8.23, 6.97 (AA′BB′, each 2H, 2 × Ar-2H), 7.23 (m, 3H, 3 × Ar-H), 7.40 (t, 1H, 7-H), 7.52 (t, 1H, Ar-H), 7.60 (d, 1H, 8-H), 12.62 (s, 1H, NH) |
| **9e** | 132–134 | 96 | $C_{21}H_{18}N_6O$ | 2.90 (s, 3H, 5-$CH_3$), 4.62 (t, 2H, $-OCH_2CH_2-$), 5.15 (t, 2H, $-OCH_2CH_2-$), 6.88 (d, 1H, 6-H), 8.27, 7.00 (AA′BB′, each 2H, 2 × Ar-2H), 7.10 (t, 1H, 7-H), 7.65 (t, 1H, Ar-H), 7.68 (t, 1H, Ar-H), 7.79 (d, 1H, 8-H), 8.14 (d, 1H, Ar-H), 8.34 (d, 1H, Ar-H) |
| **9f** | 79–81 | 98 | $C_{20}H_{25}N_5O$ | 2.31 (s, 3H, $-NCH_3$), 2.54 (brs, 8H, $-CH_2CH_2NCH_2CH_2-$), 2.67 (s, 3H ,8-$CH_3$), 2.86 (t, J = 5.8, 2H, $-NCH_2$), 4.18 (t, J = 5.8, 2H, $-CH_2O-$), 6.87 (t, J = 6.9, 1H, 6-H), 7.01, 8.21 (AA′BB′, each 2H, J = 8.8, 2 × Ar-2H), 7.24 (d, J = 6.9, 1H, 7-H), 8.42 (d, J = 6.9, 1H, 5-H) |
| **9g** | 147–149 | 82 | $C_{18}H_{17}N_5O$ | 2.69 (s, 3H, 8-$CH_3$), 4.30 (t, 2H, $-OCH_2CH_2-$), 4.40 (t, 2H, $-OCH_2CH_2-$), 6.90 (t, 1H, 6-H), 8.28, 7.00 (AA′BB′, each 2H, 2 × Ar-2H), 7.10 (d, 2H, imidazole 4′-H, 5′-H), 7.26 (d, 1H, 7-H), 7.64 (s, 1H, imidazole 2′-H), 8.45 (d, 1H, 5-H) $^{13}$C-NMR (100.6 MHz, $CDCl_3$): δ 163.24 (C=N, triazole), 159.33 (C–O, Bn), 152.15 (C=N, triazole), 129.23 (3 CH, pyridine), 128.04, 126.79, 125.74, 124.73, 113.25 (arom C or CH), 114.56 (4 CH, Bn), 67.19 ($CH_2$–O), 46.39 ($CH_2$–N), 14.02 ($CH_3$). |
| **9h** | 154–156 | 85 | $C_{17}H_{14}N_6O$ | 2.69 (s, 3H, 8-$CH_3$), 4.42 (t, 2H, $-OCH_2CH_2-$), 4.63 (t, 2H, $-OCH_2CH_2-$), 6.90 (t, 1H, 6-H), 8.27, 6.99 (AA′BB′, each 2H, 2 × Ar-2H), 7.26 (d, 1H, 7-H), 7.99 (s, 1H, triazole 3′-H), 8.22 (s, 1H, triazole 5′-H), 8.45 (d, 1H, 5-H) |
| **9i** | 210–212 | 97 | $C_{22}H_{19}N_5OS$ | 2.82 (s, 3H, 8-$CH_3$), 3.72 (t, 2H, $-OCH_2CH_2-$), 4.38 (t, 2H, $-OCH_2CH_2-$), 7.16 (t, 1H, 6-H), 8.12, 7.20 (AA′BB′, each 2H, 2 × Ar-2H), 7.25 (m, 3H, Ar-H), 7.29 (d, 1H, 7-H), 7.40 (m, 1H, Ar-H), 8.74 (d, 1H, 5-H) |
| **9j** | 146–148 | 96 | $C_{21}H_{18}N_6O$ | 2.88 (s, 3H, 8-$CH_3$), 4.06 (t, 2H, $-OCH_2CH_2-$), 4.32 (t, 2H, $-OCH_2CH_2-$), 6.86 (t, 1H, 6-H), 8.29, 7.08 (AA′BB′, each 2H, 2 × Ar-2H), 7.32 (d, 1H, 7-H), 7.47 (t, 1H, Ar-H), 7.66 (t, 1H, Ar-H), 7.81 (d, 1H, Ar-H), 8.14 (d, 1H, Ar-H), 8.34 (d, 1H, 5-H) |
| **9k** | 149–151 | 82 | $C_{20}H_{21}N_5O$ | 1.80 (m, 2H, $-OCH_{2CH_2}CH_2CH_2-$), 2.04 (m, 2H, $-OCH_2CH_2CH_2CH_2-$), 2.84 (s, 3H, 5-$CH_3$), 4.06 (m, 4H, $-OCH_2CH_2CH_2CH_2-$), 6.83 (d, 1H, 6-H), 6.97 (m, 3H, Ar-2H, imidazole 5′-H), 7.09 (s, 1H, imidazole 2′-H), 7.41 (t, 1H, 7-H), 7.53 (s,1H, imidazole 4′-H), 7.63 (d, 1H, 8-H), 8.27 (d, 2H, Ar-2H) |
| **9l** | 106–108 | 80 | $C_{19}H_{20}N_6O$ | 1.83 (m, 2H, $-OCH_{2CH_2}CH_2CH_2-$), 2.14 (m, 2H, $-OCH_2CH_2CH_2CH_2-$), 2.88 (s, 3H, 5-$CH_3$), 4.06 (t, 2H, $-OCH_2CH_2CH_2CH_2-$), 4.29 (t, 2H, $-OCH_2CH_2CH_2CH_2-$), 6.81 (d, 1H, 6-H), 8.25, 6.99 (AA′BB′, each 2H, 2 × Ar-2H), 7.41 (t, 1H, 7-H), 7.61 (d, 1H, 8-H), 7.96 (s, 1H, triazole 3′-H), 8.11 (s, 1H, triazole 5′-H) |
| **9m** | 151–153 | 76 | $C_{28}H_{24}N_6O_2$ | 2.07 (s, 6H, 2 × 8-$CH_3$), 4.52 (t, 2H, $-OCH_2CH_2-$), 4.84 (t, 2H, $-OCH_2CH_2-$), 6.91 (t, 2H, 2 × 6-H), 7.60 (m, 2H, 2 × 7H), 8.28, 7.12 (AA′BB′, each 4H, 4 × Ar-2H), 8.45 (d, 2H, 2 × 5-H) |

ing details of these structures, we can not yet reveal the dependency of the activity of the studied molecules on their structures. This result suggested that the 1,2,4-triazolo[1,5-a]pyridine structure has growth inhibitory activities against tested fungi, especially against *C. albicans*, worthy of further investigation.

**Table 2.** Antifungal activities of 2-aryl-1,2,4-triazolo[1,5-a]pyridines **9a-m**.

| Compound | *C. albicans 7374* | *T. rubrum 6180* |
|---|---|---|
| | $MIC_{50}$ [mg/mL] | $MIC_{50}$ [mg/mL] |
| **9a** | 32 | 128 |
| **9b** | >256 | >256 |
| **9c** | 128 | 256 |
| **9d** | 256 | >256 |
| **9e** | 128 | 256 |
| **9f** | 32 | 128 |
| **9g** | 64 | >256 |
| **9h** | 128 | >256 |
| **9i** | 256 | >256 |
| **9j** | 32 | 256 |
| **9k** | 64 | 256 |
| **9l** | 64 | 256 |
| **9m** | 128 | 256 |
| Fluconazole | 32 | 256 |
| Amphotericin B | 4 | 64 |

*We gratefully acknowledge Professor Yan Jie and Mrs. Ruan Pin for performing antifungal tests.*

## Experimental

### Materials

Most chemicals were purchased from Acros Organics Company (J & K chemical LTD, Shanghai, P. R. China). Melting points were determined by BUCHI Melting Point B-450 (Büchi Labortechnik, Flawil, Switzerland). $^1$H-NMR spectra were recorded in $CDCl_3$ on Bruker Avance DMX 400 using TMS as an internal standard (Bruker, Billerica, MA, USA). The IR spectra were recorded in potassium bromide on Bruker Victor 20. Mass spectral data were obtained by electron ionization on GC-HP-5989 spectrometer (Hewlett Packard, Palo Alto, CA, USA).

### Preparation of compounds 1–5

#### *Ethyl N-mesityloxysulfinyloxyacetimidate **3***

A solution of mesityl sulfochloridite **1** (5.0 g, 48 mL) and triethylamine (6.75 mL) in DMF (15 mL) was cooled to −5°C. Ethyl *N*-hydroxyacetimidate **2** (10.6 g, 48.5 mmol) was added followed by triethylamine (0.5 mL). The mixture was stirred at room temperature for 0.5 h and then poured into ice-water. The white precipitation was filtered and washed with ice-water. Yield 70.1%; m.p.: 56–58°C.

#### *Mesityl aminooxy sulfinate **4***

To a solution of **3** (7.3 g, 26 mmol) in 1.4-dioxane (5.6 mL), 70% $HClO_4$ (3.4 mL) was added drop-wise at −5°C. After stirring for 20 min, the solution was poured into ice-water. The white precipitation was filtered and washed until neutral. The product **4** was used for the next step immediately.

#### *1-Amino-methylpyridinium mesityl sulfite **5***

The above mentioned product was extracted with dichloromethane. The dichloromethane layer was added directly to a solution of methylpyridine in dichloromethane (6 mL). The mixture was stirred at room temperature for one hour, then ethyl ether was added to obtain the white precipitation. The product **5** was filtered and recrystallized by ethanol-acetic ether. Yield of two steps: 80%; m.p.: 121–123°C.

#### *Synthesis of 2-(4-benzyloxy-phenyl)-5-methyl[1,2,4]triazolo[1,5-a]pyridine **6a***

A solution of **5a** [7] (2.46 g, 8 mmol) and 4-benzyloxybenzonitrile (2.10 g, 10 mmol) in ethanol (15 mL) was cooled in ice-water, then 2N KOH (4.2 mL) was added drop-wise. After being raised to room temperature, the mixture was stirred for further 24 h and then concentrated under pressure to remove ethanol. The residual liquid was extracted with $CHCl_3$. The $CHCl_3$ layer was dried over anhydrous $Na_2SO_4$ and evaporated to dryness. The residue was purified by column chromatography to afford compound **6a**. Yield: 33.5%; MS (m/z): 315 [$M^+$]; IR (KBr): 1640, 1615, 1540, 1240.

#### *2-(4-Benzyloxy-phenyl)-8-methyl[1,2,4]triazolo[1,5-a]pyridine **6b***

Compound **6b** was prepared by the procedure described above. Yield: 39.9%; MS (m/z): 315 [$M^+$].

#### *Synthesis of 4-(5-methyl[1,2,4]triazolo[1,5-a]pyridine-2-yl)phenol **7a***

To a solution of **6a** (2.8 g) in DMF (150 mL), 10% Pd-C (0.28 g) was added. The product was obtained by hydrogenolysis reaction. Yield: 90%; MS (m/z): 225 [$M^+$]; IR (KBr): 3450, 1620, 1595, 1500.

#### *4-(5-Methyl[1,2,4]triazolo[1,5-a]pyridine-2-yl)phenol **7b***

Compound **7b** was prepared by the procedure described above. Yield: 91%; MS (m/z): 225 [$M^+$].

#### *Synthesis of 2-[2-bromoethoxyl)phenyl]-5-methyl[1,2,4]triazolo[1,5-a]pyridine **8a***

A solution of **7a** (1.50 g, 6 mmol), 1,2-dibromoethane (33.8 g, 180 mmol), NaOH (2.4 g, 60 mmol) in 3.6 mL water and tetrabutylammonium iodide (0.2 g) was refluxed for 5 h, then cooled to room temperature. The solution was extracted with $CH_2Cl_2$. The $CH_2Cl_2$ layer was washed with water and dried over anhydrous $Na_2SO_4$, then evaporated to dryness. The residue was purified by column chromatography to afford compound **8a**. Yield: 85.8%; MS (m/z): 331 ([$M^+$]:332).

#### *2-[4-(2-Bromoethoxyl)pheny]-8-methyl[1,2,4]triazolo[1,5-a]pyridine **8b** and 2-[4-(4-bromo-butoxyl)pheny]-8-methyl[1,2,4]triazolo[1,5-a]pyridine **8c***

Compounds **8b** and **8c** were prepared by the procedure described above. **8b**: Yield: 87.9%; MS (m/z): 331([$M^+$:332]). **8c**: Yield: 58.6%; MS (m/z): 359 [$M^+$].

#### *2-[4-(2-(Morpholin-4-yl)ethoxy)phenyl]-5-methyl[1,2,4]triazolo[1,5-a]pyridine **9a***

A mixture of **8a** (0.25 g, 0.75 mmol) and morpholine (0.66 mL, 7.5 mmol) was dissolved in $CHCl_3$ (50 mL), stirred at room temperature for 6 h, then concentrated under pressure to remove $CHCl_3$. The residual liquid was extracted with $CHCl_3$. The $CHCl_3$ layer was washed with water, dried over anhydrous $Na_2SO_4$ and

evaporated to dryness. The residue was purified by column chromatography to afford compound **9a**.

Compounds **9d, 9f, 9i** were prepared by the procedure described above.

### *Synthesis of 2-[4-(2-(imidazol-1-yl)ethoxy)phenyl]-5-methyl[1,2,4]triazolo[1,5-a]pyridine **9b***

To a well-stirred suspension of 60% NaH (0.67 g, 16.7 mmol) in dry DMF (5 mL), imidazole (0.68 g, 10 mmol) was added slowly. The resulting mixture was stirred for 20 min and then allowed to rise to 50 °C. A solution of **8a** (1.33 g, 4 mmol) in THF (20 mL) was added drop-wise for 3–5 h. After the reaction was completed, ice-water was added. The mixture was extracted with $CHCl_3$. The $CHCl_3$ layer was dried over anhydrous $Na_2SO_4$ and evaporated to dryness. The residue was purified by column chromatography to afford compound **9b**.

Compounds **9c, 9e, 9g, 9h, 9j, 9k, 9l, 9m** were prepared by the procedure described above.

### Antifungal assays

The method used for this test was adopted form Muanza *et al*. (1994) [9] and Mitscher *et al*. (1972) [10] with minor modifications. The test was done in duplicates, and the positive antifungal results were based on no growth compared to solvent control. $MIC_{50}$ was defined as the lowest concentration which resulted in a culture with turbidity less than or equal to the 50% inhibition. The compounds under study were dissolved in DMSO to a concentration of 256 μg/mL and serially diluted in a two-fold manner in the medium (SD broth). The final concentration of antifungal agents was between 0.25 and 256 μg/mL. The inoculated sizes contained $2 \times 10^8$ CFU/mL. MIC values were read after 7 days at 28 °C.

## References

[1] K. Yigal, A. H. Christopher, *Curr Opin Chem Biol.* **1997**, *1*, 176 – 182.

[2] M. C. Mar, G. R. Felix, *Lancet Infect Dis.* **2002**, *2*, 550 – 563.

[3] C. O. Frank., J. P. Alistair, *Trends Microbiol.* **2003**, *11*, 272 – 279.

[4] G. L. Zhang, Y. Z. Hu, *J. Chem. Res. Synop.* **2002**, 560 – 561.

[5] L. Tao, Y. Z. Hu, *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2411 – 2413.

[6] C. Sheng, W. Zhang, H. Ji, Y. Zhou, Y. Song, J. Zhu, S. Yang, *Journal of Chinese Pharmaceutical Sciences* **2002**, *11*, 5 – 9.C.

[7] Y. Tamara, K. Minamikawa, K. Sumoto, *J. Org. Chem.* **1973**, *38*, 1239 – 1241.

[8] C. Zhou, A. Hassner, *Carbohydr Res.* **2001**, *333*, 313 – 326.

[9] D. N. Muanza, B. W. Kim, K. L. Eluer, L. Williams, *Int. J. Pharmacog.* **1994**, *32*, 337 – 345.

[10] L. Mitscher, R. Luc, M. S. Bathala, W. Wu, J. L. Beal, *Lloydia* **1972**, *35*, 157 – 166.