## **Supplementary Information**

Discovery of potential antifungal triazoles: Design, synthesis, biological evaluation, and preliminary antifungal mechanism exploration

Yuan Zhang,§<sup>a</sup> Guri L V Damu,§#<sup>a</sup> Sheng-Feng Cui,§<sup>a</sup> Jia-Li Mi,<sup>b</sup> Vijai Kumar Reddy Tangadanchu#<sup>a</sup> and Cheng-He Zhou\*<sup>a</sup>

<sup>a</sup>Institute of Bioorganic & Medicinal Chemistry, Key Laboratory of Applied Chemistry of Chongqing Municipality, School of Chemistry and Chemical Engineering, Southwest University, Chongqing 400715, PR China.

<sup>b</sup>People's Hospital of Suining, Sichuan 629000, PR China.

§ These authors contributed equally to this work.

# Postdoctoral fellow from Indian Institute of Chemical Technology (IICT), India.

\* Corresponding author: zhouch@swu.edu.cn; Tel.: +86-23-68254967; Fax: +86-23-68254967.

## **1 Experimental Protocols**

#### **1.1 General Methods**

TLC analysis was done using pre-coated silica gel plates. FT-IR spectra were carried out on Bruker RFS100/S spectrophotometer (Bio-Rad, Cambridge, MA, USA) using KBr pellets in the 400–4000 cm<sup>-1</sup> range. <sup>1</sup>H NMR and <sup>13</sup>C NMR spectra were recorded on a Bruker AV 300 spectrometer using TMS as an internal standard. The following abbreviations were used to designate groups: Ph = phenyl. The chemical shifts were reported in parts per million (ppm), the coupling constants (*J*) are expressed in hertz (Hz) and signals were described as singlet (s), doublet (d), triplet (t) as well as multiplet (m). The mass spectra (MS) were recorded on LCMS-2010A and the high-resolution mass spectra (HRMS) were recorded on an IonSpec FT–CR mass spectrometer with ESI resource. The fluorescence spectra were recorded on F-7000 Spectrofluorimeter (Hitachi, Tokyo, Japan) equipped with 1.0 cm quartz cells and UV spectra were recorded at room temperature on a TU-2450 spectrophotometer (Puxi Analytic Instrument Ltd. of Beijing, China) equipped with 1.0 cm quartz cells.

Calf thymus DNA (Sigma Chemical Co., St. Louis, MO) was used without further purification, and its stock solution was prepared by dissolving an appropriate amount of DNA in doubly distilled water. The solution was allowed to stand overnight and store at 4 °C in the dark for about a week. The concentration of DNA in stock solution was determined by UV absorption at 260 nm using a molar absorption coefficient  $\xi_{260} = 6600 \text{ L} \text{ mol}^{-1} \text{ cm}^{-1}$  (expressed as molarity of phosphate groups) by Bouguer-Lambert-Beer law. The purity of DNA was checked by monitoring the ratio of the absorbance at 260 nm to that at 280 nm. The solution gave a ratio of > 1.8 at A<sub>260</sub>/A<sub>280</sub>, which indicated that DNA was sufficiently free from protein. NR stock solutions were adjusted with Tris-HCl buffer solution (pH = 7.4), which was prepared by mixing and diluting Tris solution with HCl solution. All chemicals were of analytical reagent grade, and doubly distilled water was used throughout. HSA was dissolved in Tris-HCl buffer solution (0.05 M Tris, 0.15 M NaCl, pH = 7.4). Sample masses were weighed on a microbalance with a resolution of 0.1 mg. All other chemicals and solvents were commercially available, and were used without further purification.

The pH of the solution was determined by using a Sartorius PB-10 pH meter (Sartorius Scientific Instrument (Beijing) Co., Ltd., P.R. China). Trishydroxymethylaminomethane (Tris), HCl, NaCl, and NaOH were analytical grade products and used as supplied. All other chemicals were purchased from Chongqing chemical Co. and, unless otherwise indicated, were of analytical grade. The water used for experiments was doubly distilled.

#### **1.2 Biological Assay Procedures**

Minimal inhibitory concentration (MIC,  $\mu$ g/mL) is defined as the lowest concentration of target compounds that completely inhibited the growth of fungi, by means of standard two-fold serial dilution method in 96-well microtest plates according to the National Committee for Clinical Laboratory Standards (NCCLS). The tested microorganism strains were provided by the School of Pharmaceutical Sciences, Southwest University and the College of Pharmacy, Third Military Medical University. Fluconazole and miconazole were used as control drugs. DMSO with inoculation bacterial not medicine was used as positive control to ensure that the solvent had no effect on bacteria growth. All the fungi growth was monitored visually and spectrophotometrically, and the experiments were performed in triplicate.

## 2 General Procedure and Spectral Data for Some Representative Compounds

Synthesis of 1-(2-(3,4-dichlorobenzyloxy)-2-(2,4-difluorophenyl) ethyl)-1H-1,2,4-triazole (**5b**). To a solution of compound **4** (2.4 mmol) in dry THF (20 mL) was added NaH (2.4 mmol). After the resulting mixture was stirred at 0 °C for 0.5 h. 1,2-dichloro-4-(chloromethyl)benzene (2.4 mmol) was added. The reaction system was stirred at room temperature for 6 h and the excess hydride was decomposed with a small amount of methanol. After evaporation to dryness under reduced pressure, the crude residue was suspended with water and extracted with methylene chloride. The organic layer was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, filtered and evaporated to dryness under reduced pressure. The crude residue was purified with chromatography on a silica-gel column by using solvents (petroleum ether/ethyl acetate: 1/1, V/V) as the eluent to afford the desired compound **5b** as white solid, m.p.: 89–90 °C, yield 33.4 %. IR (KBr): 3105, 2898 (aliphatic C–H), 1620, 1501 (aromatic frame), 1460, 1426, 1373, 1275,1142, 1108, 964, 854, 803, 679, 610 cm<sup>-1</sup>; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  : 8.04 (s, 1H, triazole-3-*H*), 7.91 (s, 1H, triazole-5-*H*), 7.32–7.24 (m, 1H, 2,4-2FPh-3-*H*, m, 2H, 2,4-2FPh-5,6-*H*), 7.19 (s, 1H, 3,4-2ClPh-2-*H*), 6.88–6.78 (m, 2H, 3,4-2ClPh-5,6-*H*), 4.99–4.95 (m, 2H, OCH<sub>2</sub>), 4.35–4.31 (m, 2H, triazole-CH<sub>2</sub>), 4.15–4.13 (m, 1H, 2,4-2FPhC*H*) pm; <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>)  $\delta$  : 164.8, 162.4, 162.4, 161.5, 161.3, 158.9, 152.0, 144.2, 137.0, 132.4, 131.9, 130.5, 126.8, 112.3, 104.7, 104.4, 73.1, 69.7, 54.0 pm; HRMS (ESI) calcd. for C<sub>17</sub>H<sub>14</sub>Cl<sub>2</sub>F<sub>2</sub>N<sub>2</sub>O [M+H]<sup>+</sup>, 384.0482; found, 384.0481.

Synthesis of 4-(2,4-difluorobenzyl)-1-(2-(2,4-difluorobenzyl oxy)-2-(2,4-difluorophenyl)ethyl)-1H-1,2,4-triazol-4-ium chloride (**6c**). A mixture of 1-(2-((2,4-difluorobenzyl)oxy)-2-(2,4-difluorophenyl)ethyl)-1H-1,2,4-triazole **5c** (1.6 mmol) in acetonitrile (20 mL) in the presence of potassium carbonate (1.3 mmol) was stirred at 80 °C for 0.5 h, and then 1-(chloromethyl)-2,4-difluorobenzene (2.0 mmol) was added dropwise and the reaction continued for 8 h at the same temperature. After the reaction came to the end, acetonitrile solvent was removed and treated with water, and the organic layer of ethyl acetate was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, filtered and evaporated to dryness under reduced pressure. The crude residue was purified with chromatography on a silica-gel column by using solvent (petroleum ether/ethyl acetate: 1/1, V/V) as the eluent to give the desired compound **6c** as white solid, m.p.: 101–102 °C, yield: 85.4%. IR (KBr) : 3418.8, 3072.0 (aromatic C-H), 2951.8 (aliphatic C-H), 2799.6, 1892.3, 1773.8, 1618.54, 1505.5 (aromatic frame), 1455.6, 1432.1, 1283.2, 1141.7, 1023.5, 1066.8, 979.8, 856.4,819.4, 790.1, 709.6, 674.7 cm<sup>-1</sup>; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  11.34(s, 1H, triazole-3-*H*), 8.74(s, 1H, triazole-5-*H*), 8.18–8.10 (m, 1H, 2,4-2FPh-3-*H*), 7.50–7.42(m, 1H, 2,4-2FPh-3-*H*), 7.22–7.17(m, 1H, 2,4-2FPh-3-*H*), 6.98–6.57(m, 6H, 2,4-2FPh-5,6-*H*), 5.97 (s, 2H, 2,4-2FPh-GH<sub>2</sub>), 5.20–5.16 (m, 1H, chiral-*H*), 4.78–4.71(m, 1H, triazole-CH<sub>2</sub>), 4.51–4.31(m, 3H, OCH<sub>2</sub>, triaozle-CH<sub>2</sub>) ppm; <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>)  $\delta$ : 165.7, 165.0, 164.8, 163.0, 162.8, 162.3, 162.2, 161.7, 161.5, 159.7, 159.3, 158.8, 143.6, 133.7, 133.6, 131.7, 131.7, 129.3, 129.2, 119.4, 119.3, 118.8, 118.6, 116.1, 115.8, 112.8, 112.5, 112.3, 111.4, 111.2, 104.6, 104.4, 104.0, 103.6, 103.3, 70.9, 64.6, 56.4, 45.3 ppm; HRMS (ESI) calcd. for C<sub>24</sub>H<sub>18</sub>F<sub>6</sub>N<sub>2</sub>O [M]<sup>+</sup>, 478.1349; found, 478.1344.

*Synthesis of 1-(2-(2,4-difluorophenyl)-2-propoxyethyl)-1H-1,2,4-triazole* (**7a**). To a solution of triazole alcohol **4** (2.4 mmol) in dry THF (20 mL) was added sodium hydride (2.4 mmol). After the resulting mixture was stirred at 0 °C for 0.5 h, the 1-bromopropane (2.4 mmol) was added. The mixture was continuously stirred at room temperature for 6 h and the excess hydride was decomposed with a small amount of methyl alcohol. After evaporation to dryness under reduced pressure, the crude residue was suspended in water and extracted with methylene chloride. The organic layer was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, filtered and evaporated to dryness under reduced pressure. The crude residue was purified with chromatography on a silica gel column by using solvents (petroleum ether/ethyl acetate: 1/1, V/V) as eluent to afford the desired compound **7a** as yellow liquid, yield: 75.6 %. IR (KBr): 3052 (aromatic C–H), 2964 (aliphatic C–H), 2926 (aliphatic C–H), 2877.6 (aliphatic C–H), 1618, 1503 (aromatic frame), 1457, 1430, 1351, 1273, 1209, 1139, 1103, 1054, 1011, 962, 852, 817, 747, 705, 680, cm<sup>-1</sup>; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>):  $\delta$  8.15 (s, 1H, triazole-3-*H*), 7.93 (s, 1H, triazole-5-*H*), 7.37–7.30 (m, 1H, 2,4-2FPh-3-*H*), 6.94–6.81 (m, 2H, 2,4-2FPh-5,6-*H*), 4.96–4.92 (m, 1H, chiral-*H*), 4.39–4.29 (m, 2H, OCH<sub>2</sub>), 3.36–3.29 (m, 1H, triazole-CH<sub>2</sub>), 3.19–3.12 (m, 1H, triazole-CH<sub>2</sub>), 1.52-1.46 (m, 2H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 0.81 (t, 3H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>) ppm; <sup>13</sup> C NMR (75 MHz, CDCl<sub>3</sub>):  $\delta$  164.42, 161.11, 158.78, 151.57, 144.14, 128.62, 121.22, 111.80, 104.43, 104.09, 103.75, 73.44, 71.41, 54.36, 22.65, 10.38 ppm; HRMS (ESI) calcd. for C<sub>13</sub>H<sub>15</sub>F<sub>2</sub>N<sub>3</sub>O [M+H]<sup>+</sup>, 268.1261; found, 268.1262.

### 3 Molecular Modeling of Compound 5b with CYP51 or HSA



Fig.S1 Stereoview of conformation of compound 5b docked in CYP51



Fig.S2 Overall structure of HSA with binding of ligand  $\mathbf{5b}$ 



Fig.S3 Hydrophobic pocket of HSA with ligand 5b



Fig.S4 Hydrogen bond interaction of compound 5b with alanine



Fig.S5 HSA-target stick and line model

# **4 Figures for DNA Interaction**



Fig. S6 The plot of  $A^{0}/(A-A^{0})$  versus 1/[compound **5b**].



Fig. S7 The plot of  $A^{0}/(A-A^{0})$  versus 1/[compound 9c].



Fig. S8 UV absorption spectra of NR in the presence of DNA at pH 7.4 and room temperature.  $c(NR) = 2 \times 10^{-5} \text{ mol/L}$ , and  $c(DNA) = 0-3.84 \times 10^{-5} \text{ mol/L}$  for curves a-i respectively at an increment of  $0.48 \times 10^{-5} \text{ mol/L}$ .



Fig. S9 UV absorption spectra of the competitive reaction between 5b and neutral red with DNA.  $c(DNA) = 4.17 \times 10^{-5} \text{ mol/L}$ ,  $c(NR) = 2 \times 10^{-5} \text{ mol/L}$ , and  $c(\text{compound 5b}) = 0-2.3 \times 10^{-5} \text{ mol/L}$  for curves a–i respectively at an increment of  $0.29 \times 10^{-5} \text{ mol/L}$ . Inset: absorption spectra of the system with the increasing concentration of 5b in the wavelength range of 400–600 nm absorption spectra of competitive reaction between compound 5b and NR with DNA.



**Fig. S10** UV absorption spectra of the competitive reaction between **9c** and neutral red with DNA.  $c(DNA) = 4.17 \times 10^{-5} \text{ mol/L}$ ,  $c(NR) = 2 \times 10^{-5} \text{ mol/L}$ , and  $c(\text{compound } 9c) = 0-2.1 \times 10^{-5} \text{ mol/L}$  for curves a-i respectively at an increment of  $0.26 \times 10^{-5} \text{ mol/L}$ . Inset: Absorption spectra of the system with the increasing concentration of **9c** in the wavelength range of 350–600 nm absorption spectra of competitive reaction between compound **9c** and NR with DNA.

## 5 Some Representative Spectra for Compound's Structure

#### 5.1 Spectra of Compound 5b



## <sup>13</sup>C NMR Spectrum









### 5.2 Spectra of Compound 5c



<sup>13</sup>C NMR Spectrum













#### <sup>13</sup>C NMR Spectrum





<sup>5.4</sup> Spectra of Compound 7a



### <sup>13</sup>C NMR Spectrum





## 5.5 Spectra of Compound 8c

### <sup>1</sup>H NMR Spectrum





### **MS Spectrum**



**IR Spectrum** 



### 5.6 Spectra of Compound 9c

