DESCRIPTION

ANTI-*CANDIDA ALBICANS* PIPERAZINE DERIVATIVE, PREPARATION METHOD THEREFOR AND APPLICATION THEREOF

FIELD OF THE INVENTION

The present invention relates to the field of compound synthesis, in particular to an anti-*Candida albicans* piperazine derivative, a preparation method therefor and application thereof.

BACKGROUND OF THE INVENTION

Candida albicans, a fungal disease widely spread in humans, is now one of the most important pathogens of hospital-acquired infection. The fungus can infect and colonize a wide range of microbial environments in the human body, including blood vessels, mucosal surfaces and major internal organs. As a conditional pathogen, *Candida albicans* can not only cause thrush and vaginitis, but also result in severe systemic infection and lead to high mortality in immunodeficiency patients. At present, the most effective treatment and prevention of *Candida albicans* infection is the local and systemic use of azole antifungal drugs, which can directly kill the bacteria; however, with the widespread use of azole drugs, drug resistance is also getting worse. For this reason, it is very valuable to develop a drug with a new antibacterial strategy to treat *Candida albicans* infection.

Candida albicans has a very special characteristic, i.e., it is yeast-mycelium biphasic. The morphological transition of *Candida albicans* from a yeast form to a mycelium form is an important factor for *Candida albicans* to infect cells. *Candida albicans* first adheres in a free yeast form, and promotes tissue invasion through morphological transition; patients infected with *Candida albicans* usually have tissues infected due to penetration of mycelial pathogens. Defective strains with morphological transition mutations during infection are non-toxic, and morphological

transition is very important for the pathogenicity of *Candida albicans*. Therefore, we use the importance of adhesion and morphological transition of *Candida albicans* in its pathogenic effects to screen for new drugs that can inhibit this strain.

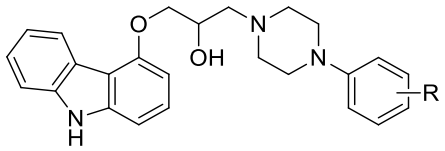
CONTENTS OF THE INVENTION

The primary purpose of the present invention is to overcome the disadvantages and deficiencies of the prior art and provide an anti-*Candida albicans* piperazine derivative.

Another object of the present invention is to provide a method for preparing the above-mentioned anti-*Candida albicans* piperazine derivative.

Yet another object of the present invention is to provide application of the above-mentioned anti-*Candida albicans* piperazine derivative.

The objects of the present invention are achieved through the following technical solution: The structural formula of the anti-*Candida albicans* piperazine derivative is as represented by formula I:



Formula I

where R is 2-X, 4-X or 4-CX_3, X is a halogen element, including F, Cl, Br and I, and 2 and 4 indicate positions where a substituent is located.

The R is preferably 4-CF_3, 2-Cl, 2-Br or 4-Br.

The method for preparing the anti-*Candida albicans* piperazine derivative comprises the following steps:

(1) 4-Hydroxycarbazole and epichlorohydrin are mixed uniformly, then a NaOH solution is added dropwise at -10°C to 10°C, and the obtained mixture is heated to 60°C to continue the reaction, with TLC used to detect the progress of the reaction; after the reaction, an appropriate amount of water is added, and ethyl acetate is used for extraction; the organic phases are combined, washed with water, dried and filtered;

the filtrate is recovered, and the solvent is evaporated to dryness to obtain an epoxide;

(2) the epoxide and piperazine containing different substituents are dissolved in isopropanol and undergo a reflux reaction at 0°C to 80°C for 4-6 h, with the progress of the reaction detected by TLC; after the reaction, the reaction system is cooled and the solvent is evaporated to dryness, such that an anti-*Candida albicans* piperazine derivative is obtained through purification and recrystallization; wherein the amount of the piperazine containing different substituents is determined according to the molar ratio of piperazine to 4-hydroxycarbazole, which is 1:1.

The molar ratio of epichlorohydrin to 4-hydroxycarbazole in step (1) is not less than 1, so that 4-hydroxycarbazole can be fully utilized; preferably, the molar ratio of epichlorohydrin to 4-hydroxycarbazole is 1:1 to 2:1; more preferably, the molar ratio of epichlorohydrin to 4-hydroxycarbazole is 1.2:1.

The concentration of the NaOH solution step (1) is preferably 40% by mass.

The molar amount of NaOH in step (1) is preferably twice the molar amount of 4-hydroxycarbazole.

The dropping temperature in step (1) is preferably 0°C.

The specific steps of detecting the progress of the reaction by TLC in step (1) are preferably as follows: Silica gel GF254 is used for thin layer chromatography, with a mixed solution of petroleum ether and ethyl acetate in a volume ratio of 7:1 to 3:1 used as a developing agent; the detection is performed until a raw material point disappears.

The water in step (1) is used for suspension dispersion.

The drying in step (1) is preferably carried out with anhydrous magnesium sulfate.

The piperazine containing different substituents in step (2) is preferably 1-(4-trifluoromethylphenyl)piperazine, 1-(2-chlorophenyl)piperazine, 1-(2-bromophenyl)piperazine, or 1-(4-bromophenyl)piperazine.

The reflux reaction in step (2) is preferably carried out at 80°C for 6 h.

The specific steps of detecting the progress of the reaction by TLC in step (2)

are preferably as follows: Silica gel GF254 is used for thin layer chromatography, with a mixed solution of petroleum ether and ethyl acetate in a volume ratio of 4:1 used as a developing agent; the detection is performed until a piperazine compound point disappears.

The purification in step (2) is preferably carried out by column chromatography.

The filler of the column chromatography is preferably silica gel of 200-300 meshes.

The column chromatography is preferably eluted with a mixture of petroleum ether and ethyl acetate in a volume ratio of 6:1.

The recrystallization in step (2) is preferably carried out with 95% ethanol by mass.

The anti-*Candida albicans* piperazine derivative can be applied to the preparation of an anti-*Candida albicans* infection drug.

The present invention has the following advantages and effects with respect to the prior art:

(1) The inventor of this application has identified in the previous research that the quorum-sensing signal of the diffusible signal factor (DSF) and its derivatives can strongly interfere with the yeast-mycelium transition of *Candida albicans*. The present study also successfully synthesized and screened out compounds that can inhibit the adhesion, mycelial morphological transition and pathogenicity of *Candida albicans*. Besides, these compounds are less toxic and do not affect the growth of *Candida albicans* and human cells. These characteristics may promote the development of new antifungal drugs.

(2) The yield of the preparation method provided by the present invention is as high as about 75%.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 shows the synthesis process of the piperazine derivative, where the letter a

represents sodium hydroxide.

Fig. 2 shows the detected effects of the piperazine derivative on *Candida albicans* adhering to polystyrene, wherein Fig. (A) shows the detected effects of 45 synthetic piperazine derivatives at a final concentration of 100 μM on the cell adhesion of *Candida albicans*, and Fig. (B) shows the inhibition rates of 6 compounds of 22, 24, 25, 26, 27 and 28 at different concentrations of 12.5-200 μM, fluconazole being used as a positive control, the data showing the average results of 8 biological replicates, the error bar reflecting the standard deviation.

Fig. 3 shows the detected effects of piperazine derivatives on the formation of *Candida albicans* mycelium, wherein Fig. (A) shows the inhibition rates of 45 synthetic piperazine derivatives at a final concentration of 100 μM on the formation of *Candida albicans* mycelium, Fig. (B) shows the inhibition effects of 6 compounds of 22, 24, 25, 26, 27 and 28 at different concentrations of 50-200 μM, and Fig. (C) is a photomicrograph of inhibition of mycelial formation by DMSO, fluconazole, compounds 22, 24, 25, 26, 27 and 28 at a final concentration of 100 μM, the data showing the average results of 3 biological experiments, the error bar reflecting the standard deviation.

Fig. 4 shows the detected effects of piperazine derivatives on the pathogenicity of *Candida albicans*, wherein Fig. A shows the detected effects of 45 synthetic piperazine derivatives at a final concentration of 100 μM on the cytotoxicity of A549 cells, Fig. B shows the detected effects of 45 synthetic piperazine derivatives at a final concentration of 100 μM on the cytotoxicity of *Candida albicans*, and Fig. C shows the detected effects of the compounds 25, 26, 27 and 28 at different concentrations of 3.125-100 μM on the cytotoxicity of *Candida albicans*; with the LDH release amount measured to detect the cytotoxicity, for detecting the cytotoxicity of *Candida albicans*, the LDH release amount of a group to which DMSO was added was taken as 100%, and then the LDH release ratio of other groups to which the piperazine derivative was added was regulated; the data show the average results of 4 biological replicates, and the error bar reflects the standard

deviation.

Fig. 5 shows the detected effects of piperazine derivatives on the growth rate of *Candida albicans*, fluconazole being used as a positive control, the data showing the average results of 3 biological replicates, the error bar reflecting the standard deviation.

DETAILED DESCRIPTION OF THE EMBODIMENTS

The present invention is described in further detail below with reference to examples and accompanying drawings, but the embodiments of the present invention are not limited thereto.

Example 1: Synthesis of piperazine derivatives

1. As shown in Fig. 1, piperazine derivatives were synthesized as follows:

(1) First, 4-hydroxyindole, 4-hydroxycarbazole or 4-hydroxyacetophenone were respectively mixed with epichlorohydrin (in a molar ratio of 1:1.2); a sodium hydroxide aqueous solution in a mass (g)-volume (mL) ratio of 40% was dropwise added at 0°C (the amount of NaOH was twice the moles of 4-hydroxyindole, 4-hydroxycarbazole or 4-hydroxyacetophenone); after the dropwise addition, the temperature was raised to 60°C to allow a reaction to occur to obtain the corresponding epoxy compound, wherein TLC (thin-layer chromatography, using silica gel GF254 as the material) was used to detect the progress of the reaction until a raw material point disappeared, with a mixed solution of petroleum ether and ethyl acetate in a volume ratio of 7:1 used as a developing agent; after the reaction, water was added, and ethyl acetate was used for extraction; the organic phases were combined, washed with water, dried with anhydrous magnesium sulfate, and filtered; the filtrate was recovered, and the solvent was evaporated to dryness;

(2) then, the obtained products respectively reacted at reflux with the piperazine containing different substituents (the dosage of the piperazine was consistent with the moles of 4-hydroxyindole, 4-hydroxycarbazole or 4-hydroxyacetophenone, with the specific compounds as shown in Table 1) in isopropanol at a high temperature (80°C)

for 6 h, wherein the progress of the reaction was detected by TLC until a piperazine point disappeared, with a mixed solution of petroleum ether and ethyl acetate in a volume ratio of 4:1 used as a developing agent; after the reaction, the reaction system was cooled and the solvent was evaporated to dryness; after purification by column chromatography (using a mixture of petroleum ether and ethyl acetate in a volume ratio of 6:1 for elution, and silica gel of 200-300 meshes as the filler), 95% ethanol by mass was used for recrystallization to obtain compounds 1 to 45 (as shown in Table 2). Identified by NMR and mass spectrometry, the compounds were shown to have the same structure as the designed one. In the activity experiment, all samples were dissolved in DMSO for standby. The yield of the compounds obtained by this method was about 75%.

Table 1

Compound	Starting material	Piperazine containing different substituents		Compound	Starting material	Piperazine containing different substituents
1	4-hydroxyindole	1-(2-methoxyphenyl)piperazine		24	4-hydroxycarbazole	1-(2-methyl-6-ethylphenyl)piperazine
2		1-(4-methoxyphenyl)piperazine		25		1-(4-trifluoromethylphenyl)piperazine
3		1-(2,5-dimethoxyphenyl)piperazine		26		1-(2-chlorophenyl)piperazine
4		1-(3,4-dimethoxyphenyl)piperazine		27		1-(2-bromophenyl)piperazine

5		1-(2,5-diethoxyphenyl)piperazine	28		1-(4-bromophenyl)piperazine
6		1-(2,4-dimethoxy-5-chlorophenyl)piperazine	29		1-(3,4-dichlorophenyl)piperazine
7		1-(4-methylphenyl)piperazine	30	4-hydroxyacetophenone	1-(2-methoxyphenyl)piperazine
8		1-(2,4-dimethylphenyl)piperazine	31		1-(4-methoxyphenyl)piperazine
9		1-(2-chlorophenyl)piperazine	32		1-(2,4-dimethoxyphenyl)piperazine
10		1-(2-bromophenyl)piperazine	33		1-(2,5-dimethoxyphenyl)piperazine
11		1-(4-bromophenyl)piperazine	34		1-(3,4-dimethoxyphenyl)piperazine
12		1-(3,4-dichlorophenyl)piperazine	35		1-(2-ethoxyphenyl)piperazine
13	4-hydroxycarbazole	1-(2-methoxyphenyl)piperazine	36		1-(2,5-diethoxyphenyl)piperazine

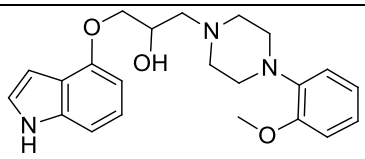
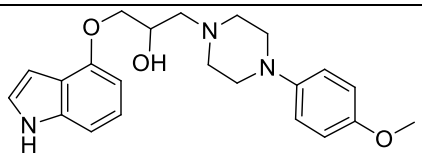
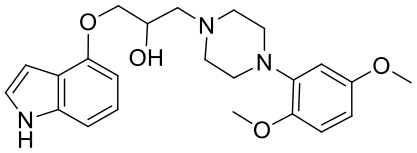
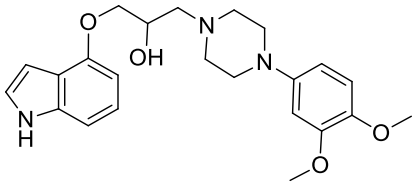
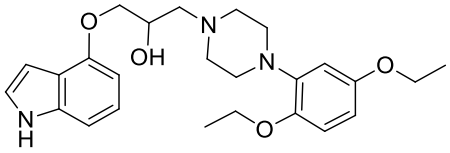
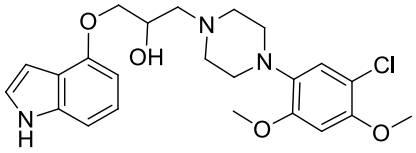
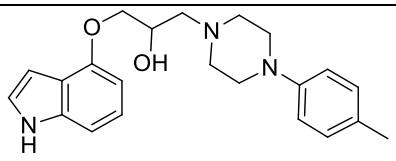
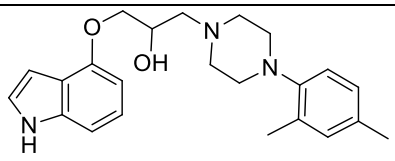
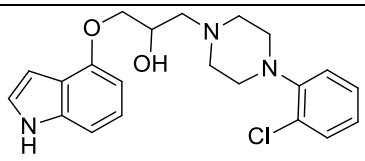
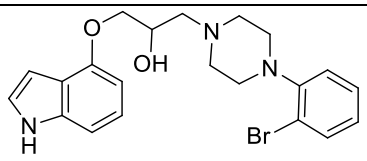
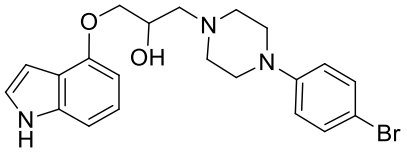
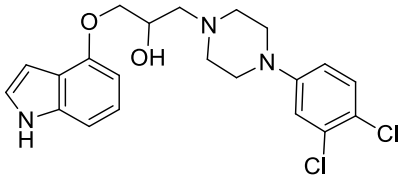
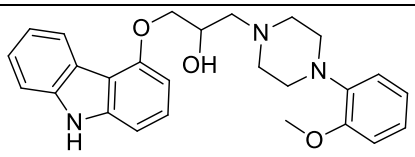
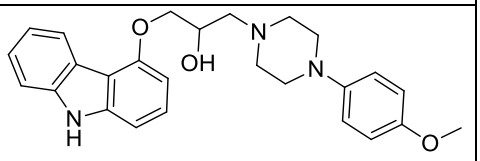
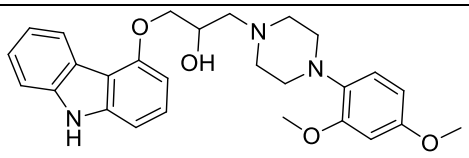
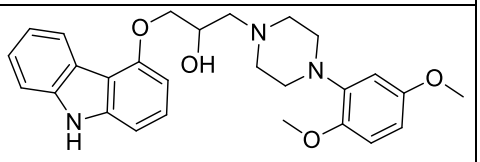
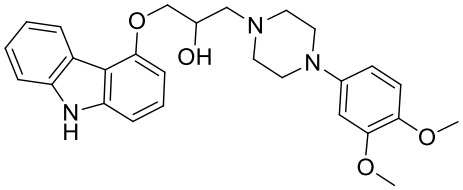
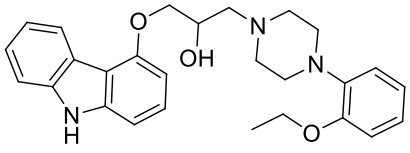
14		1-(4-methoxyphenyl)piperazine	37		1-(2,4-dimethoxy-5-chlorophenyl) piperazine
15		1-(2,4-dimethoxyphenyl)piperazine	38		1-(4-methylphenyl)piperazine
16		1-(2,5-dimethoxyphenyl)piperazine	39		1-(2,3-dimethylphenyl)piperazine
17		1-(3,4-dimethoxyphenyl)piperazine	40		1-(2-methyl-6-ethylphenyl)piperazine
18		1-(2-ethoxyphenyl)piperazine	41		1-(4-trifluoromethylphenyl)piperazine
19		1-(2,5-diethoxyphenyl)piperazine	42		1-(2-chlorophenyl)piperazine
20		1-(2,4-dimethoxy-5-chlorophenyl)piperazine	43		1-(2-bromophenyl)piperazine
21		1-(4-methylphenyl)piperazine	44		1-(4-bromophenyl)piperazine
22		1-(2,3-dimethylphenyl)piperazine	45		1-(3,4-dichlorophenyl)piperazine

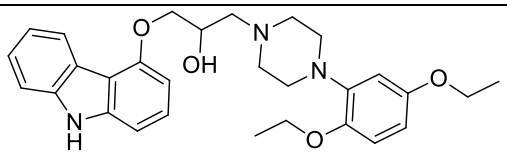
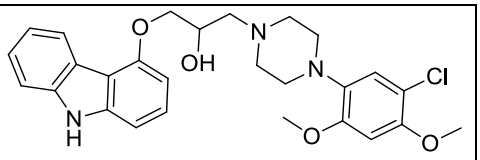
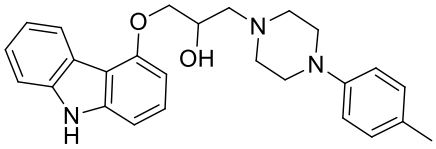
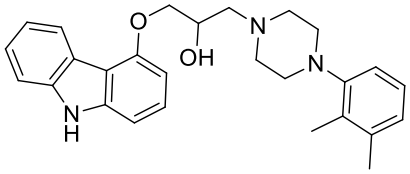
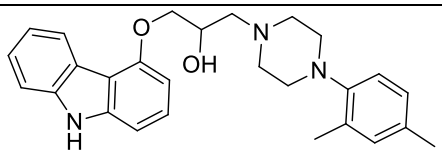
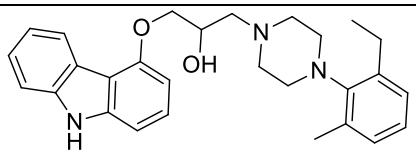
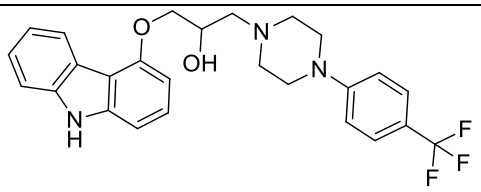
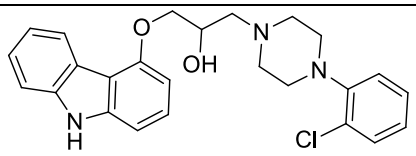
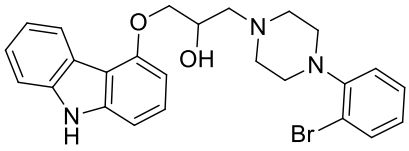
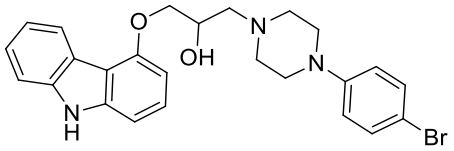
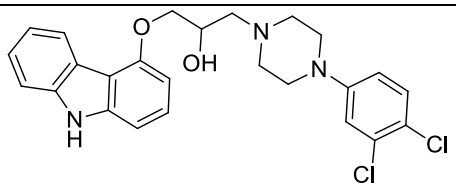
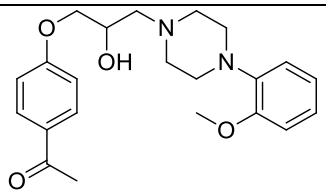
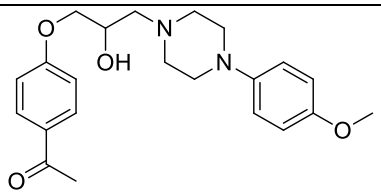
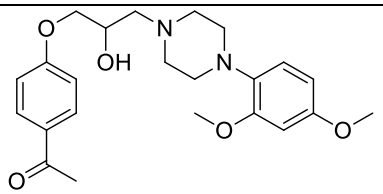
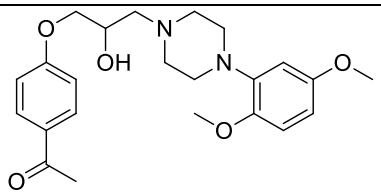
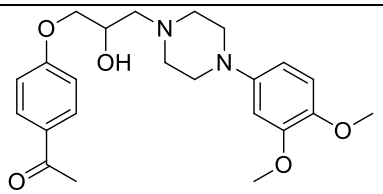
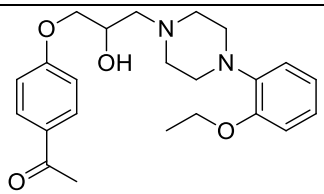
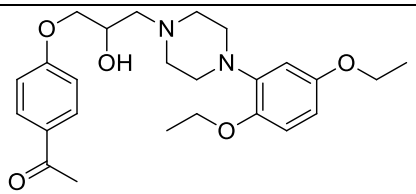
23		1-(2,4-dimethylphenyl)piperazine			

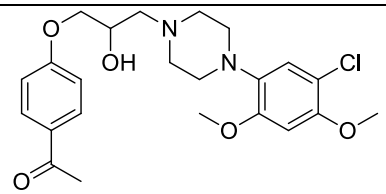
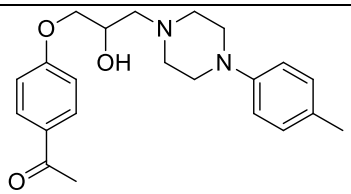
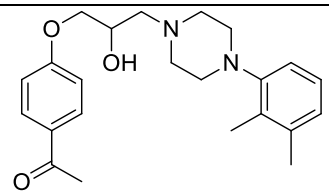
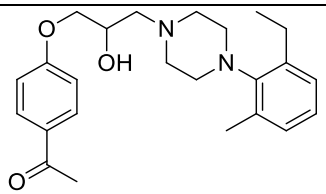
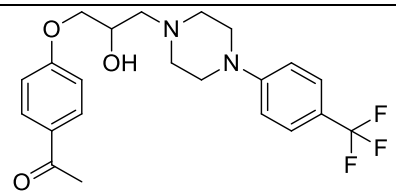
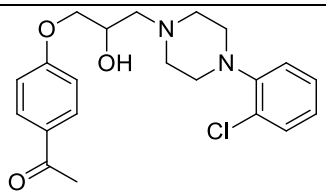
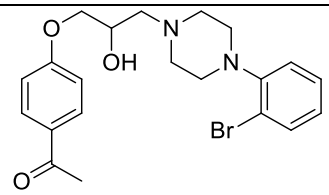
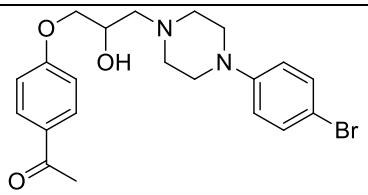
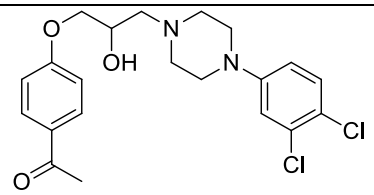
Note: Piperazine containing different substituents in Table 1 can be synthesized according to “Ge, Z. Q; Ji, Q. G.; Chen, C. Y.; Liao, Q.; Wu, H. L.; Liu, X. F.; Huang, Y. R.; Yuan, L. J.; Liao, F., Synthesis and biological evaluation of novel 3-substituted amino-4-hydroxylcoumarin derivatives as chitin synthase inhibitors and antifungal agents, *Journal of Enzyme Inhibition & Medicinal Chemistry*, 2016, 31 (2), 219-228”.

For example, compound 25 was synthesized as follows: 9.16 g (0.05 mol) of 4-hydroxycarbazole was added to 5.55 g (0.06 mol) of epichlorohydrin, and then 10 mL of a 40% (w/v) NaOH solution was added dropwise at 0°C, with the dropping completed in about 1 h; the temperature was raised to 60°C to allow a reaction to proceed for several hours, with the progress of the reaction detected by TLC; after the reaction, an appropriate amount of water was added, and ethyl acetate was used for extraction; the organic phases were combined, washed with water, and dried with anhydrous magnesium sulfate; the obtained substance was filtered, the filtrate was recovered, and the solvent was evaporated to dryness to obtain about 15 g of a brick red oily substance, which was a crude extract of 4-(ethylene oxide-2-yl)methoxy-9H-carbazole with no need for purification; the obtained epoxide and 11.5 g (0.05 mol) of 1-(4-trifluoromethylphenyl)piperazine were dissolved in an appropriate amount of isopropanol and underwent a reaction at reflux for 6 h, with the progress of the reaction detected by TLC; after the reaction, the reaction system was cooled and the solvent was evaporated to dryness; the resulting substance was purified by column chromatography (using a mixture of petroleum ether and ethyl acetate in a ratio of 6:1 for elution) and recrystallized with 95% ethanol to obtain about 17.50 g of compound 25 with a yield of 75%.

Table 2

Serial number	Compound structure	Serial number	Compound structure
1		2	
3		4	
5		6	
7		8	
9		10	
11		12	
13		14	
15		16	
17		18	

19		20	
21		22	
23		24	
25		26	
27		28	
29		30	
31		32	
33		34	
35		36	

37		38	
39		40	
41		42	
43		44	
45			

Example 2: Effect detection test

1. Detection test

(1) Adhesion test

Candida albicans SC5314 strain (ATCC, USA) was cultured overnight in a GMM nutrient solution (composed of 6.7 g/L amino acid-free yeast nitrogen source (YNB) and 0.2% glucose, referring to the method in the literature “A novel DSF-like signal from *Burkholderia cenocepacia* interferes with *Candida albicans* morphological transition”); the OD_{600} value was adjusted to 0.5, the compounds at a certain final concentration were sequentially added and mixed well, and the obtained mixture was added to a 96-well plate (polystyrene material) at 200 μL/well; the culture plate was left to incubate at 37°C for 4 h, the supernatant was discarded, and 50 μL of crystal violet at a concentration of 0.5% (m/v) was added to each well for staining at room

temperature for 45 min; the crystal violet was discarded, and deionized water of ice was used to wash 10 times; the crystal violet was dissolved in 200 μL of 75% ethanol by volume, and the resulting solution was left at room temperature for 30 min, with the OD_{590} value measured with a microplate reader.

(2) Mycelium formation test

Candida albicans SC5314 strain was cultured in a GMM nutrient solution at 30°C to an OD_{600} value of 2.0, and diluted 20 times with the GMM nutrient solution; the compound was added to 0.5 mL of the diluted bacterial solution according to a certain final concentration, mixed by shaking slightly, and then placed in a 37°C water bath for 4 h, with fluconazole, BDSF (*B. cenocepacia* diffusible signal factor, a freely diffusible signal molecule isolated from *Burkholderia cenocepacia* and having a chemical name of cis-2-dodecenoic acid, purchased) and DMSO used as controls; after the action, centrifugation was performed (5000 rpm, 10 min), the supernatant was discarded, 40 μL of a fresh GMM nutrient solution was added for resuspension, and the mycelial formation was observed under a Zeiss Axioplan 2 microscope.

(3) Analysis of the growth curve of *Candida albicans*

Candida albicans SC5314 strain was cultured overnight in a GMM medium at 30°C, its OD_{600} value being adjusted to 0.05, the compound being added to make its final concentration at 100 μM; the above resulting substance was added at 300 μL/well to a 10 × 10 sterile honeycomb microporous plate (suitable for use in a Bioscreen-C automatic growth curve analyzer), which was placed in the Bioscreen-C automatic growth curve analyzer at 30°C with moderate shaking, with the instrument automatically detecting the OD_{600} value of each well every half hour and continuously monitoring for 48 h.

(4) Cytotoxicity test

Cytotoxicity was detected according to the content of lactate dehydrogenase LDH released by human lung cancer A549 cells. A549 cells were cultured overnight in a 96-well plate at a concentration of 1×10^4 cells/well in a high-sugar medium DMEM containing 10% (v/v) fetal bovine serum; when the cells grew to 80% full, the medium

was discarded, and the cells were washed three times with PBS (0.01 M, pH 7.4); *Candida albicans* SC5314 strain was cultured overnight in a GMM medium containing 0.2% (w/v) glucose in a 30°C shaker; the bacteria were collected by centrifugation, washed three times with PBS, and dispersed at a concentration of 10^8 CFU/mL in a DMEM cell maintenance solution containing 1% (v/v) FBS; and a certain concentration of the compound was added; after the interaction, the resulting substance was added to a 96-well cell plate and cultured in a cell incubator for 8 h, while DMSO and a compound-free well were used as control wells.

(5) Mouse infection test

Animal tests, using BALB/c mice (purchased from Guangdong Experimental Animal Center), were performed in accordance with the regulations on the care and use of experimental animals in the health guidance of the National Institutes of Health (NIH 8023, revised in 1978). Male mice aged 6-8 weeks were randomly assigned to different groups, 8 in each group, and weighed; *Candida albicans* SC5314 was dispersed in a PBS solution at a concentration of 5×10^8 cfu/mL; compounds 27 and 28 at a final concentration of 100 μM were added to the PBS solution containing the bacteria, and the resulting solution was injected through the tail vein at a concentration of 100 μL/10 g, while 1 × PBS (pH 7.4, 0.01 M) and 100 μM fluconazole were respectively injected as negative and positive controls, respectively.

2. Experimental results

(1) Piperazine derivatives inhibiting the adhesion of *Candida albicans*

Because adhesion is the first step of *Candida albicans* infection, we first tested whether the piperazine derivatives could inhibit the adhesion of *Candida albicans* to polystyrene. As shown in Fig. 2, 11 of the 45 compounds had an adhesion inhibition rate of more than 75% (Fig. 2A), wherein the effects of compounds 22, 24 and 25-28 were particularly outstanding. We continued to test whether the inhibition was concentration-dependent, so we chose to test the inhibition effects of these compounds at concentrations of 12.5-200 μM. The results showed that the inhibition rates of these compounds at the concentration of 12.5 μM exceeded 65% (Fig. 2B), showing that the

piperazine derivatives could effectively inhibit the adhesion of *Candida albicans*.

(2) Piperazine derivatives inhibiting the mycelial formation of *Candida albicans*

The morphological transition of *Candida albicans* from the yeast form to the mycelium form is very important for the pathogenicity of *Candida albicans*. We tested the effects of these compounds on the morphological transition of *Candida albicans* in vitro. *Candida albicans* was cultured in a GMM medium on a 30°C shaker to maintain its yeast form, and the medium was diluted in a certain proportion and then cultured at 37°C to promote the formation of mycelia. With or without the compounds in the medium, after 4 h of incubation, the majority of the cells in the negative control group with DMSO formed mycelia, while most of the cells in the group with the compounds formed less mycelia (Fig. 3). Among them, the mycelial inhibition rate of 13 compounds exceeded at least 50% at the final concentration of 100 μM (Fig. 3A), and compounds 22, 24 and 25-28 inhibited the mycelial formation in a concentration-dependent manner (Fig. 3B). With the mycelial formation further observed under a microscope, the effects of compounds 22, 24, 25, 26, 27 and 28 on the mycelial formation of *Candida albicans* at a concentration of 100 μM could be seen (Fig. 3C). It can be seen that these compounds could inhibit the mycelial formation.

(3) Piperazine derivatives reducing the pathogenicity of *Candida albicans*

In order to study whether the piperazine derivatives could affect the pathogenicity of *Candida albicans*, we tested the toxicity effect of the piperazine derivatives, with and without *Candida albicans*, on cells and mice. As expected, the toxicity of *Candida albicans* to cells was reduced after the addition of some piperazine derivatives (Fig. 4). According to the LDH experimental results, when the final concentration was 100 μM, compounds 8, 23, 24, 35, 37, 38, 39, 40 and 42 had very high cytotoxicity, while other compounds had no or extremely low cytotoxicity (Fig. 4A). When *Candida albicans* was added to the cells, some piperazine derivatives at the same concentration almost completely inhibited the toxicity of *Candida albicans* (Fig. 4B). Therefore, we selected compounds 25, 26, 27 and 28 to test the inhibition effect of *Candida albicans* at a

concentration of 3.125-100 μM (Fig. 4C). Considering that compounds 22, 24 and 25-28 had better effects in inhibiting the formation of biofilm and mycelium while compounds 22 and 24 had no effect in inhibiting the cytotoxicity of *Candida albicans*, we next mainly analyzed the inhibition effect of compounds 25-28 on the cytotoxicity of *Candida albicans* at different concentrations. The results showed that none of these four compounds affected the growth efficiency of *Candida albicans* (Fig. 5), that is, they could reduce the pathogenicity of *Candida albicans* without killing fungi, which indicated that they might be used as new antifungal drugs. The results of the mouse infection test also showed that 100% of the mice died within 30 days of infection with *Candida albicans*, but the mortality rates were 29% and 14% after the addition of compounds 27 and 28, respectively (Table 3). We can see that compound 28 is one of the candidates for new anti-*Candida albicans* drugs.

Table 3. Effects of compounds on the pathogenicity of *Candida albicans* in a mouse infection model

Compound	Survival rate (%)
DMSO	0
Fluconazole	100
27	71
28	86

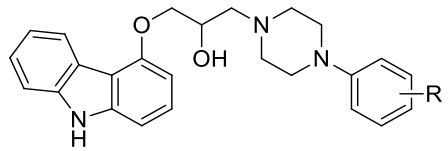
In general, we synthesized a series of piperazine derivatives and screened them for their ability against fungal pathogens. Some of these compounds show good properties of inhibiting cell adhesion, mycelial formation, and pathogenicity of *Candida albicans*, while they are non-toxic to *Candida albicans* cells and human cells. The experimental results show that some of these compounds may be developed as new anti-*Candida albicans* infection drugs.

The above examples are preferred embodiments of the present invention, but the

embodiments of the present invention are not limited thereto, and any other alterations, modifications, substitutions, combinations, and simplifications made without departing from the spirit and principle of the present invention should be equivalent replacements and included in the scope of protection of the present invention.

CLAIMS

1. An anti-*Candida albicans* piperazine derivative, characterized in that: the structural formula of the anti-*Candida albicans* piperazine derivative is as represented by formula I:



Formula I

where R is 2-X, 4-X or 4-CX_3, and X is a halogen element.

2. The anti-*Candida albicans* piperazine derivative according to claim 1, characterized in that: the R is 4-CF_3, 2-Cl, 2-Br or 4-Br.

3. A method for preparing the anti-*Candida albicans* piperazine derivative according to claim 1 or 2, characterized in that the method comprises the following steps:

(1) 4-hydroxycarbazole and epichlorohydrin are mixed uniformly, then a NaOH solution is added dropwise at -10°C to 10°C, and the obtained mixture is heated to 60°C to continue a reaction, with TLC used to detect the progress of the reaction; after the reaction, an appropriate amount of water is added, and ethyl acetate is used for extraction; the organic phases are combined, washed with water, dried and filtered; the filtrate is recovered, and the solvent is evaporated to dryness to obtain an epoxide;

(2) the epoxide and piperazine containing different substituents are dissolved in isopropanol and undergo a reflux reaction at 0°C to 80°C for 4-6 h, with the progress of the reaction detected by TLC; after the reaction, the reaction system is cooled and the solvent is evaporated to dryness, such that an anti-*Candida albicans* piperazine derivative is obtained through purification and recrystallization; wherein the amount of the piperazine containing different substituents is determined according to the molar ratio of piperazine to 4-hydroxycarbazole, which is 1:1.

4. The method for preparing the anti-*Candida albicans* piperazine derivative

according to claim 3, characterized in that:

the molar ratio of epichlorohydrin to 4-hydroxycarbazole in step (1) is not less than 1; and

the molar amount of NaOH in step (1) is twice the molar amount of 4-hydroxycarbazole.

5. The method for preparing the anti-*Candida albicans* piperazine derivative according to claim 3, characterized in that:

the dropping temperature in step (1) is 0°C;

the reflux reaction in step (2) is carried out at 80°C for 6 h; and

the drying in step (1) is carried out with anhydrous magnesium sulfate.

6. The method for preparing the anti-*Candida albicans* piperazine derivative according to claim 3, characterized in that: the piperazine containing different substituents in step (2) is 1-(4-trifluoromethylphenyl)piperazine, 1-(2-chlorophenyl)piperazine, 1-(2-bromophenyl)piperazine, or 1-(4-bromophenyl)piperazine.

7. The method for preparing the anti-*Candida albicans* piperazine derivative according to claim 3, characterized in that:

the purification in step (2) is carried out by column chromatography.

8. The method for preparing the anti-*Candida albicans* piperazine derivative according to claim 7, characterized in that:

the filler of the column chromatography is silica gel of 200-300 meshes; and

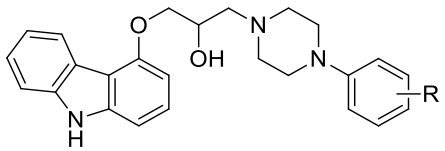
the column chromatography is eluted with a mixture of petroleum ether and ethyl acetate in a volume ratio of 6:1.

9. The method for preparing the anti-*Candida albicans* piperazine derivative according to claim 3, characterized in that: the recrystallization in step (2) is carried out with 95% ethanol by mass.

10. Application of the anti-*Candida albicans* piperazine derivative according to claim 1 or 2 to the preparation of an anti-*Candida albicans* infection drug.

ABSTRACT

The present invention discloses an anti-*Candida albicans* piperazine derivative, a preparation method therefor and application thereof. The structural formula of the piperazine derivative is as represented by formula I. The piperazine derivative has the following advantages: The piperazine derivative is compounds that can inhibit the adhesion, mycelial morphological transition and pathogenicity of *Candida albicans*; besides, these compounds are less toxic themselves and do not affect the growth of *Candida albicans* and human cells. Therefore, the piperazine derivative may be developed as a new anti-*Candida albicans* infection drug.



Formula I

Figure to be published with Abstract: **Figure 4**

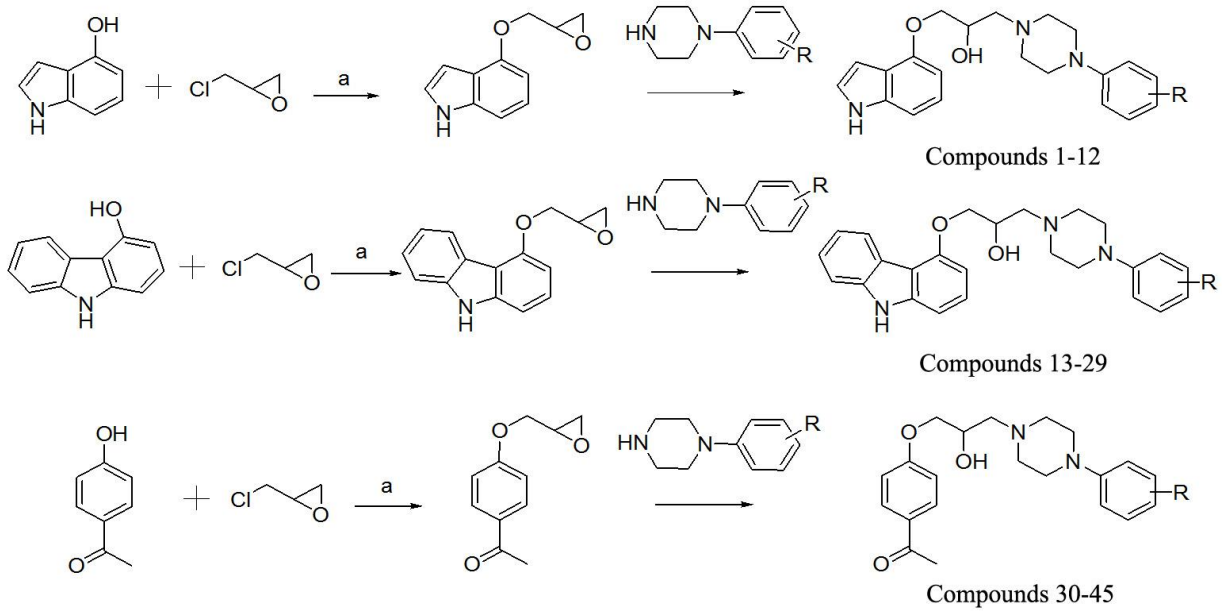

OH
Cl
a
HN
N
R
O
OH
Compounds 1-12
HO
Compounds 13-29
Compounds 30-45

Fig. 1

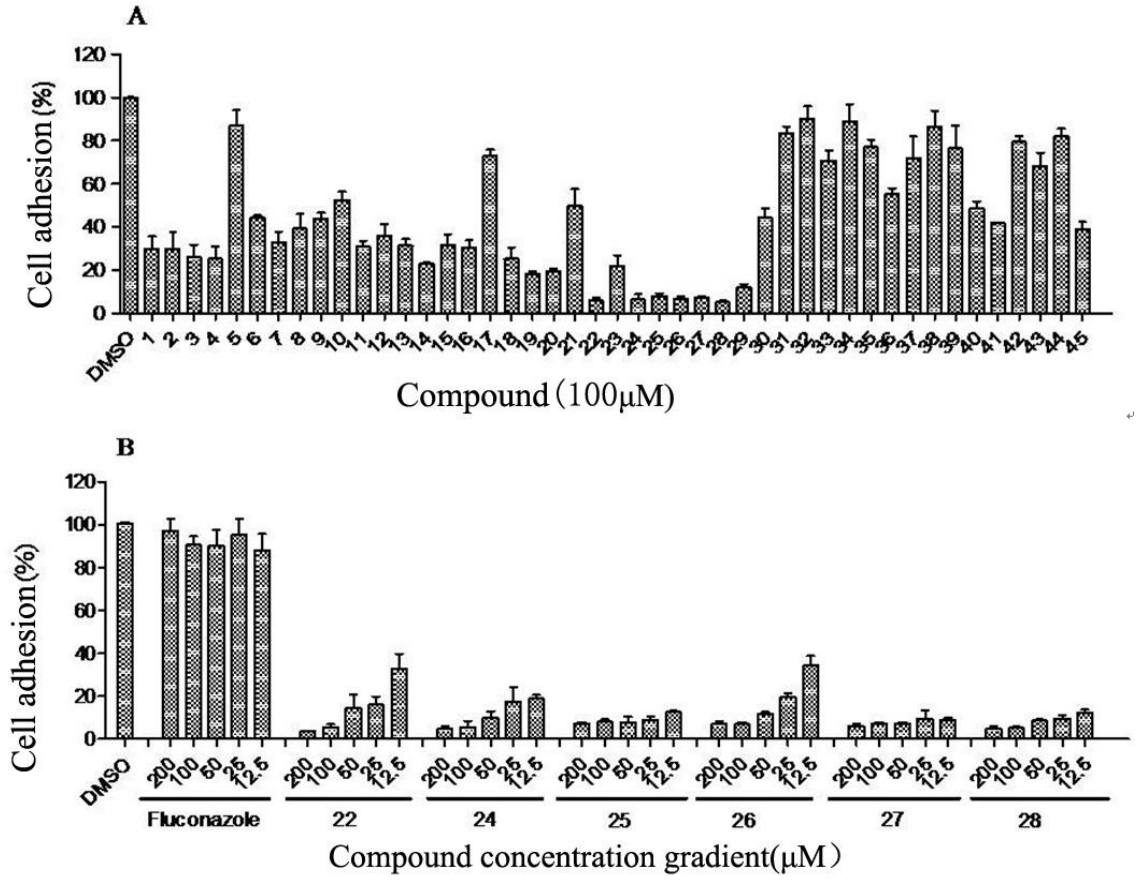
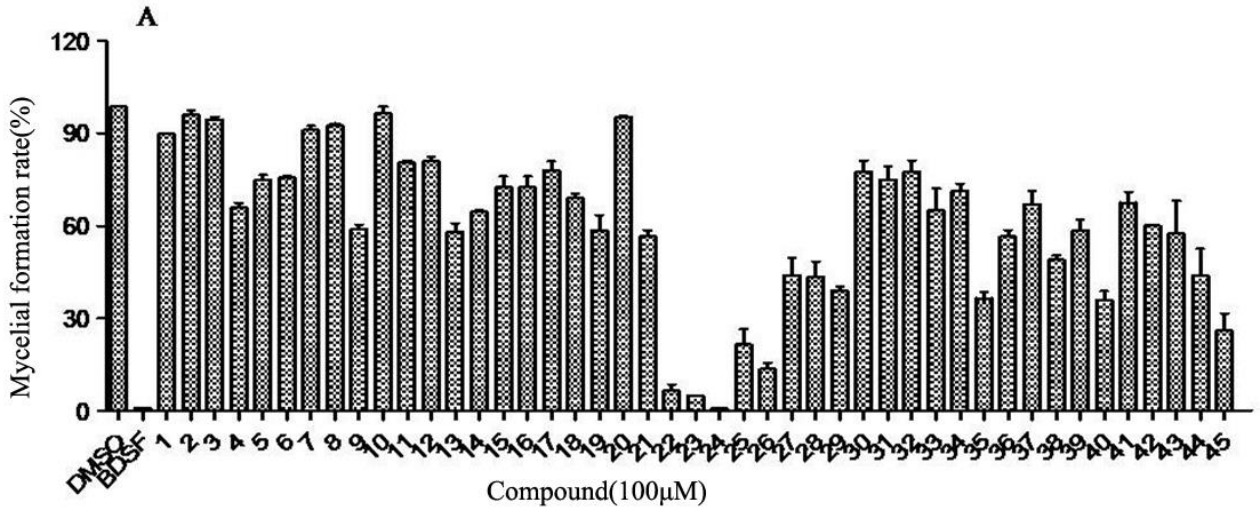
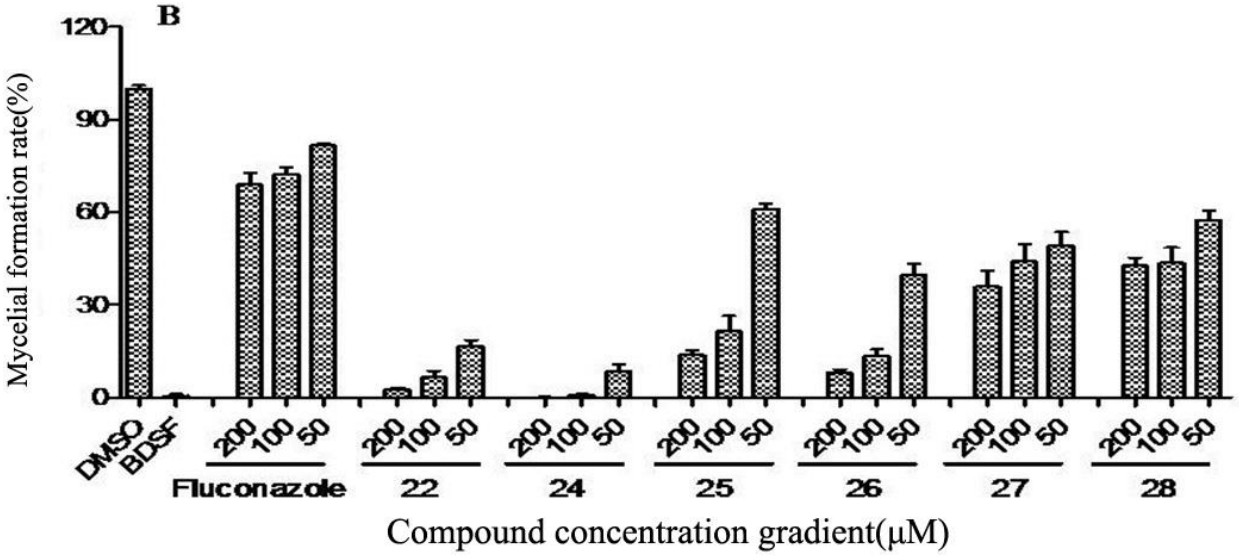

A
Cell adhesion (%)
120
100
80
60
40
20
0
DMSO
Compound (100μM)
B
Cell adhesion (%)
200
100
60
25
12.5
Fluconazole
22
24
25
26
27
28
Compound concentration gradient(μM)

Fig. 2


A
Mycelial formation rate(%)
120
90
60
30
0
DMSO
BDSF
Compound(100μM)


B
Mycelial formation rate(%)
120
90
60
30
0
DMSO
BDSF
200
100
50
Fluconazole
22
24
25
26
27
28
Compound concentration gradient(μM)

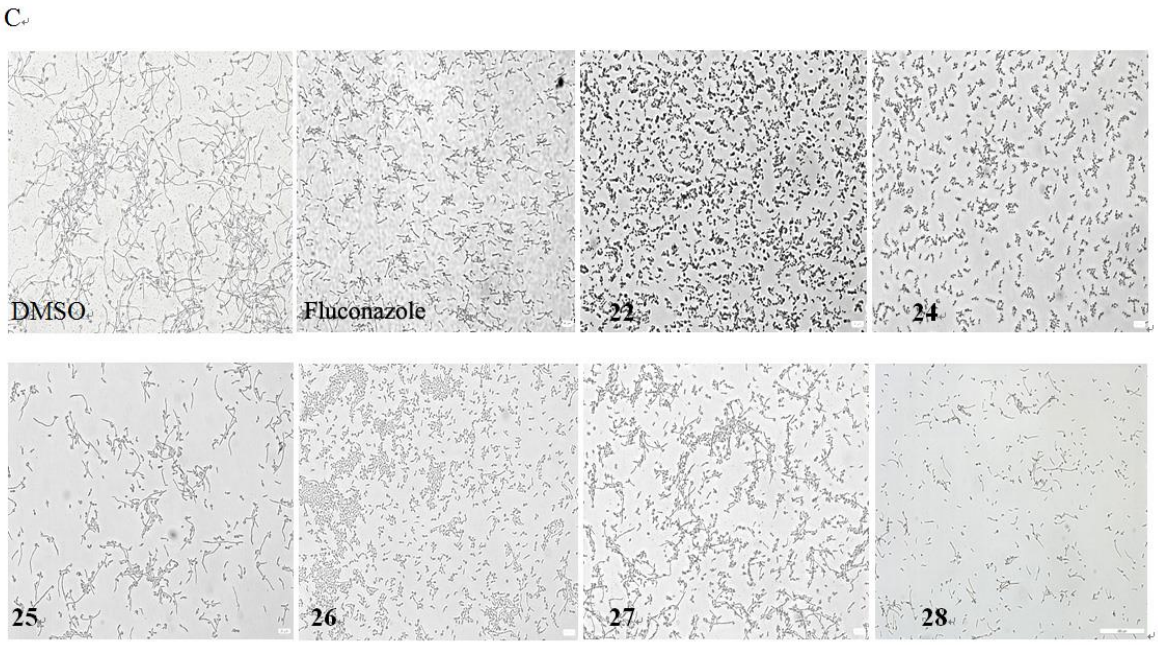

C
DMSO
Fluconazole
22
24
25
26
27
28

Fig. 3

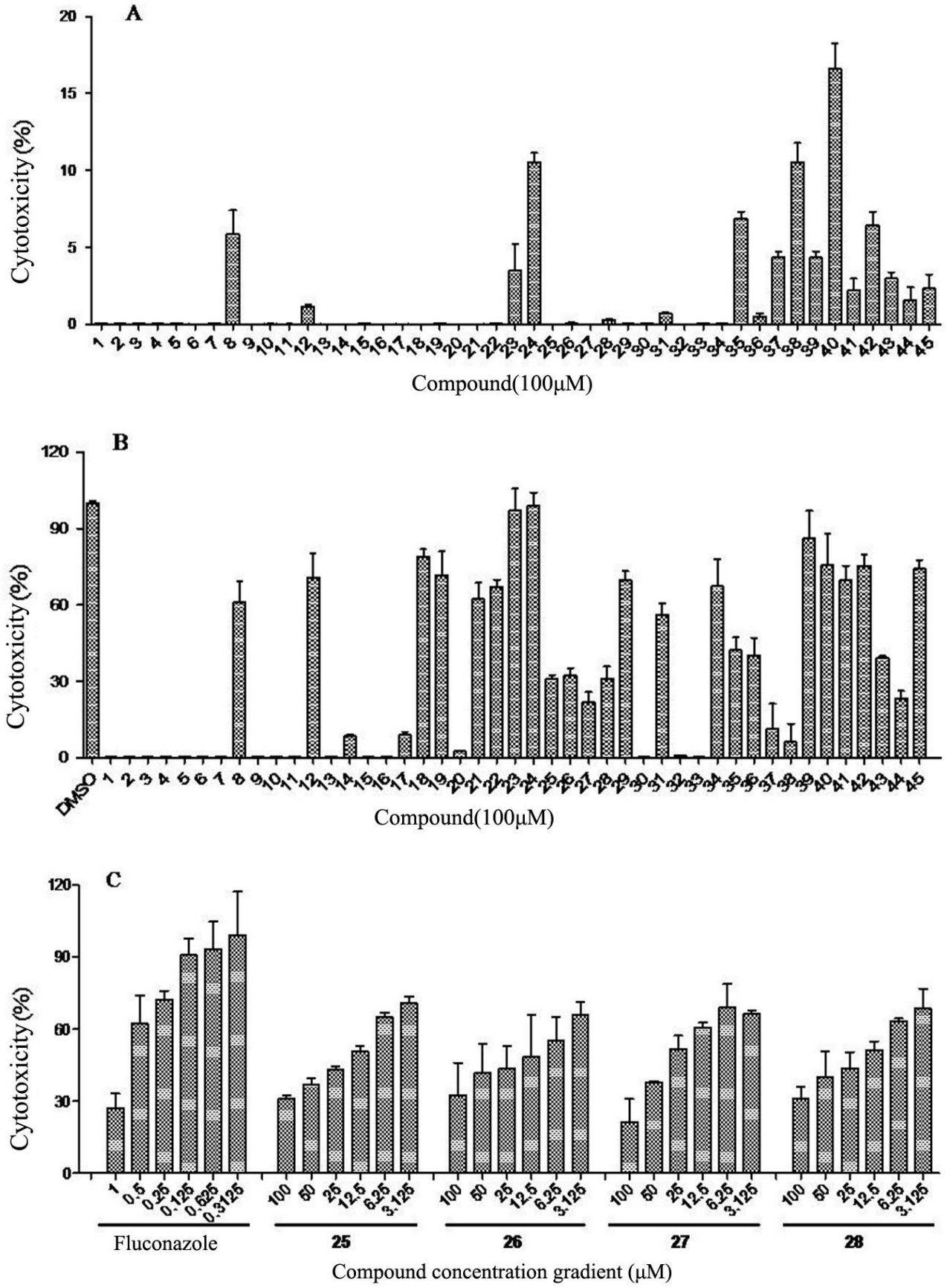

A
Cytotoxicity (%)
Compound(100μM)
B
Cytotoxicity (%)
DMSO
Compound(100μM)
C
Cytotoxicity (%)
Fluconazole
25
26
27
28
Compound concentration gradient (μM)

Fig. 4

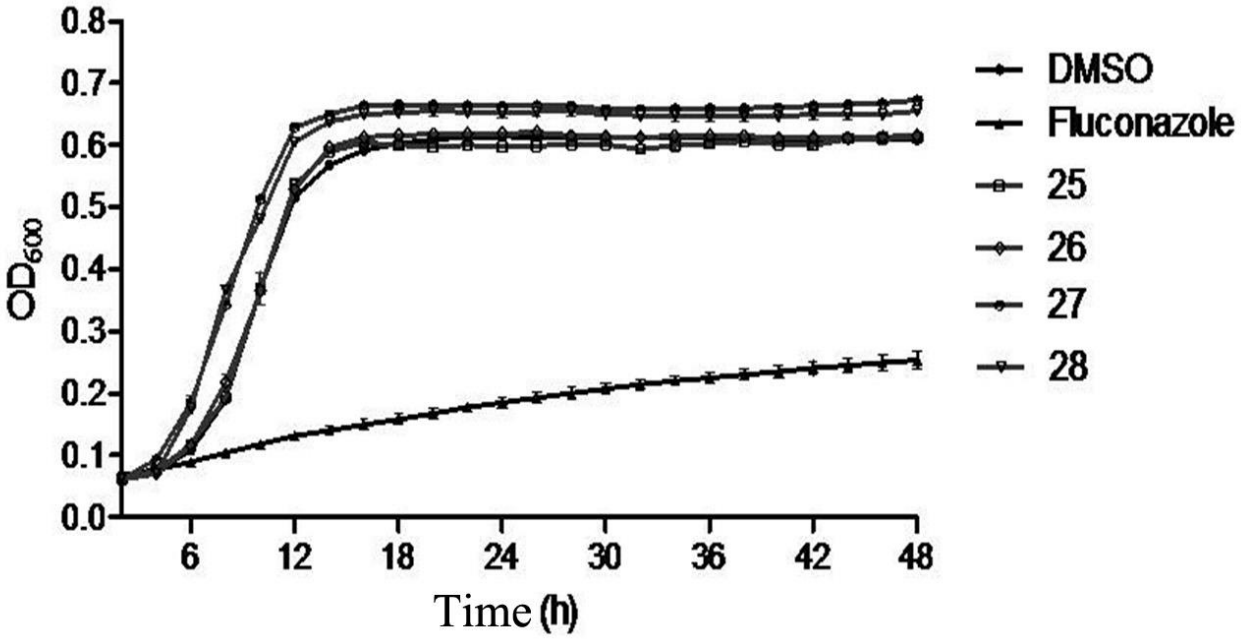

0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
OD600
6
12
18
24
30
36
42
48
Time (h)
DMSO
Fluconazole
25
26
27
28

Fig. 5