Antifungal activity of thymol against clinical isolates of fluconazole-sensitive and -resistant *Candida albicans*

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Thymol (THY) was found to have *in vitro* antifungal activity against 24 fluconazole (FLC)-resistant and 12 FLC-susceptible clinical isolates of *Candida albicans*, standard strain ATCC 10231 and one experimentally induced FLC-resistant *C. albicans* S-1. In addition, synergism was observed for clinical isolates of *C. albicans* with combinations of THY–FLC and THY–amphotericin B (AMB) evaluated by the chequerboard microdilution method. The interaction intensity was determined by spectrophotometry for the chequerboard assay, and the nature of the interactions was assessed using two non-parametric approaches [fractional inhibitory concentration index (FICI) and ΔE models]. The interaction between THY–FLC or THY–AMB in FLC-resistant and -susceptible strains of *C. albicans* showed a high percentage of synergism by the FICI method and the ΔE method. The ΔE model gave results consistent with FICI, and no antagonistic action was observed in the strains tested.

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INTRODUCTION

The dimorphic fungus *Candida albicans* is a commensal organism of the human oral, gastrointestinal, vaginal, cutaneous and mucosal surfaces. However, it is also an opportunistic fungal pathogen, causing candidiasis in individuals with an underlying pathological process or deficient state. Oropharyngeal candidiasis is a common initial presentation in patients with AIDS. In patients receiving broad-spectrum antibiotics or undergoing cancer chemotherapy, *C. albicans* can enter the bloodstream to cause serious systemic invasive disease (Pappas *et al.*, 2009). Over the last few decades, the occurrence of *Candida* infections has increased significantly, paralleling the growing numbers of immunocompromised patients. For patients, disseminated candidiasis can be a serious disease, often resulting in death (Anttila *et al.*, 1994).

Amphotericin B (AMB), a polyene fungicidal agent, has been the standard drug for the treatment of candidal infections for decades, but the toxicity of its conventional form and the cost of its lipid forms limit its use (Marchetti

et al., 2003). More recently, azole antifungal compounds, which have excellent efficacy–toxicity profiles, have emerged as the principal drugs used in the treatment of candidal infections in non-neutropenic patients. The most widely used drug in both the treatment and prevention of candidiasis is fluconazole (FLC), a member of the azole family of drugs, which targets the essential enzyme Erg11, lanosterol 14 α -demethylase, in the ergosterol biosynthetic pathway (Vanden Bossche, 1985). However, in recent years, prolonged use of FLC has contributed to the development of drug resistance in *C. albicans* and other species (Sanglard *et al.*, 2003). One selective pressure contributing to the emergence of drug resistance is the fungistatic rather than fungicidal character of FLC action (Uppuluri *et al.*, 2008). Thus, new therapeutic strategies are necessary. Improvements in the efficacy of antifungal drug therapy and reductions in toxicity may be achieved by using combination therapy (Tariq *et al.*, 1995).

Thymol (THY) [5-methyl-2-(1-methylethyl) phenol] is an essential oil, found primarily in thyme, oregano and tangerine peel (He *et al.*, 1997). Studies have shown that essential oils containing THY are widely used in medicine for their antimicrobial and disinfectant properties (Didry *et al.*, 1994; Ogaard *et al.*, 1997; Healing & Oppenheim, 1990), and also act as antibacterial additives in food and feed (Burt *et al.*, 2007). Notably, THY has been successfully

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Abbreviations: AMB, amphotericin B; ATCC, American Type Culture Collection; BI, Bliss independence; CLSI, Clinical and Laboratory Standards Institute; FICI, fractional inhibitory concentration index; FLC, fluconazole; LA, Loewe additivity; THY, thymol.

used *in vitro* against pathogenic fungi, including *Aspergillus*, *C. albicans* and moulds (Giordani *et al.*, 2004; Shin & Kim, 2004).

In this study, we assessed the *in vitro* antifungal activity of THY against clinical isolates of *C. albicans* and standard *Candida* strains. We also observed the *in vitro* interaction of combinations of drugs (THY–FLC and THY–AMB) against FLC-resistant *C. albicans* and FLC-susceptible *C. albicans* using a chequerboard microdilution assay.

METHODS

Fungal strains and materials. Twenty-two clinical isolates of FLC-resistant *C. albicans* (Quan *et al.*, 2006) and twelve clinical isolates of FLC-susceptible *C. albicans*, two clinical isolates of *C. albicans* TW 00313 (CDR1,2 overexpression) and TW 07239 (MDR1/ERG11 overexpression) (White *et al.*, 2002), and one experimentally induced FLC-resistant *C. albicans* S-1 (Deng *et al.*, 2006) were kindly provided by Y. Y. Jiang (Department of Pharmacology, School of Pharmacy, Second Military Medical University, Shanghai, PR China), T. C. White (Seattle Biomedical Research Institute, Seattle, WA, USA) and X. L. Deng (Peking University School and Hospital of Stomatology, Beijing, PR China), respectively. Four ATCC type *Candida* strains (*C. albicans* ATCC 10231, *Candida tropicalis* ATCC 13803, *Candida parapsilosis* ATCC 90018 and *Candida krusei* ATCC 6258) were acquired from the American Type Culture Collection (ATCC), Gaithersburg, MD, USA. The components of YPD broth (1% yeast extract, 2% peptone and 2% glucose) were obtained from BD Biosciences. THY ($\geq 99\%$ pure) was purchased from the China Medical Culture Collection Center (Beijing, PR China), FLC was obtained from Pfizer, and AMB were obtained from Sigma. Stock solutions of varying concentrations were made in DMSO (Sigma).

Antifungal susceptibility testing. In order to choose the appropriate range of drug concentrations for the combination studies, the MICs of THY, FLC and AMB against *C. albicans* were determined by broth microdilution using twofold serial dilutions in RPMI 1640 medium, as described by the Clinical and Laboratory Standards Institute (CLSI) (formerly the National Committee for Clinical Laboratory Standards) (approved standard M27-A) for yeasts. Three rounds of experiments were performed, and the mean MIC value was calculated. The quality control strain, *C. krusei* ATCC 6258, and the reference strain, *C. parapsilosis* ATCC 90018, were included in each batch of susceptibility tests to ensure quality. The test was carried out in 96-well flat-bottomed microtitration plates according to a published report by Quan *et al.* (2006). After agitation for 15 s, the plates were incubated at 35 °C without shaking, and readings were performed after 24 h incubation by both visual reading and optical density determination at 630 nm.

For the visual reading, the MICs of FLC were determined according to CLSI procedures, growth was graded on a scale from 0 to 4, as follows: 4 indicated no reduction in growth, 3 indicated a 25% reduction of growth, 2 indicated a 50% reduction of growth, 1 indicated a 75% reduction of growth and 0 indicated 100% reduction of growth (an optically clear well). For THY and AMB, the MIC was defined as the lowest concentration showing 100% growth suppression. We chose a 100% growth suppression level for THY based on a published study (Koşar *et al.*, 2008), and a report that showed that THY expressed strong fungicidal activity against opportunistic yeasts (Viollon & Chaumont, 1994).

For the optical density determination, each plate was shaken for 5 min and the optical density values at 630 nm for each well were

read with a microtitre plate reader. For FLC, the susceptibility end point was defined as the lowest concentration of antifungal that resulted in growth reduced by 80% compared with the control. For THY and AMB, the MIC was defined as the lowest concentration showing 100% growth suppression.

Chequerboard microdilution assay. Assays were performed on 22 clinical isolates of FLC-resistant *C. albicans* (Quan *et al.*, 2006), 12 clinical isolates of FLC-susceptible *C. albicans*, 2 clinical isolates of *C. albicans* TW 00313 (CDR1,2 overexpression) and TW 07239 (MDR1/ERG11 overexpression), 1 experimentally induced FLC-resistant *C. albicans* S-1 and *C. albicans* ATCC 10231 as mentioned above, according to the methods of the CLSI (approved standard M27-A) (NCCLS, 1997). The initial concentration of fungal suspension in RPMI 1640 medium was 10^3 c.f.u. ml⁻¹, and the final concentrations ranged from 0.016 to 16 µg ml⁻¹ for AMB, 0.015 to 512 µg ml⁻¹ for FLC and 4 to 512 µg ml⁻¹ for THY. Plates were incubated at 35 °C for 24 h, then a visual reading of the MICs was made, the OD₆₃₀ was measured and background optical densities were subtracted from that of each well. Each isolate was tested in triplicate.

In the chequerboard assay, to assess the interaction of combinations of drugs (THY–FLC and THY–AMB), the data obtained by spectrophotometry were further analysed using two non-parametric approaches, fractional inhibitory concentration index (FICI) and ΔE (Sun *et al.*, 2008), which are based on the two major interaction theories Loewe additivity (LA) and Bliss independence (BI), respectively. The LA theory is based on the idea that a drug cannot interact with itself, while the BI theory is based on the idea that two drugs act in ways that are probably independent. In the LA theory the concentrations of the drugs, alone or in combination, that produce the same effect are compared, while in the BI-based models, the estimates of the combined effect based on the effects of the individual drugs were compared with those obtained in the experiment. FICI was defined as the sum of the MIC of each drug when used in combination divided by the MIC of the drug used alone. Synergy and antagonism were defined by FICIs of ≤ 0.5 and > 4 , respectively. An FICI result of > 0.5 but ≤ 4 was considered indifferent (Quan *et al.*, 2006).

ΔE model. The ΔE model is described by the equation $I_i = (I_A + I_B) - (I_A \times I_B)$, where I_i is the predicted percentage of inhibition of the theoretical combination of drugs A and B, and I_A and I_B are the experimental percentages of inhibition of each drug acting alone. Since I is equal to $1 - E$, where E is the percentage of growth, by substitution into the former equation, the following equation is derived: $E_i = E_A \times E_B$, where E_i is the predicted percentage of growth with the theoretical combination of drugs A and B, and E_A and E_B are the experimental percentages of growth with each drug alone. An interaction is described by the difference ΔE between the predicted and measured percentages of growth at various concentrations ($\Delta E = E_{\text{predicted}} - E_{\text{measured}}$). By the non-parametric approach described by Prichard *et al.* (1993), E_A and E_B are obtained directly from the experimental data. Due to the nature of the interaction, testing with microtitre plates and a twofold dilution of either drug results in a ΔE for each drug combination. In each of the three independent experiments, the observed percentages of growth obtained from the experimental data were subtracted from the predicted percentages, and then the mean difference of three experiments was calculated. When the mean difference and the 95% confidence interval for the three replicates was positive, a statistically significant synergy was claimed. When the difference and the 95% confidence interval was negative, significant antagonism was claimed. In any other case, BI was concluded. The values obtained for each combination were depicted on the z-axis used to construct a three-dimensional plot. Peaks above and below the 0 plane indicate synergistic and antagonistic combinations, respectively, whilst the 0

plane indicates the absence of a statistically significant interaction. As the plot only shows the interactions for each separate combination of the drug concentrations, a value is needed to summarize the interaction surface. This was accomplished by calculating the sum of the percentages of all statistically significant synergistic ($\sum$ SYN) and antagonistic ($\sum$ ANT) interactions. Interactions with <100 % statistically significant interactions were considered weak, those with 100–200 % statistically significant interactions were considered moderate, and those with >200 % statistically significant interactions were considered strong (Guo *et al.*, 2008). In addition, the numbers of statistically significant synergistic and antagonistic combinations among the 96 combinations of drug concentrations tested were calculated for each strain.

RESULTS AND DISCUSSION

MIC of THY against *C. albicans*

In this experiment, the MICs of THY against *C. albicans* strains and ATCC type strains (*C. albicans* ATCC 10231, *C. tropicalis* ATCC 13803, *C. krusei* ATCC 6258 and *C. parapsilosis* ATCC 90018) were 32–256 $\mu\text{g ml}^{-1}$ and 64–128 $\mu\text{g ml}^{-1}$, respectively (Table 1). These results showed that THY has *in vitro* antifungal activity against *C. albicans*. The MICs of FLC against ATCC 6258 and ATCC 90018 were 64 and 0.5 $\mu\text{g ml}^{-1}$, respectively, and both were within the reference ranges. The MICs of AMB against ATCC 6258 and ATCC 90018 were both 1 $\mu\text{g ml}^{-1}$, and both were within the reference ranges.

Drug susceptibility in combination

FICIs of the FLC–THY combination against 34 *C. albicans* strains ranged from 0.25 to 1, and FICIs of the FLC–THY combination against *C. albicans* TW 00313, *C. albicans* TW 07239, *C. albicans* S-1 and ATCC 10231 were 1.0, 0.5, 0.5 and 0.75, respectively (Table 1). FICIs of the AMB–THY combination against 34 *C. albicans* strains ranged from 0.25 to 1.5, and FICIs of the AMB–THY combination

against *C. albicans* TW 00313, *C. albicans* TW 07239, *C. albicans* S-1 and ATCC 10231 were 0.5, 0.5, 0.5 and 1, respectively (Table 1). We did not find antagonistic interactions between FLC–THY or AMB–THY against any strains tested. This result was consistent with another report that the THY oil, and THY as well, exhibit synergism with AMB against *C. albicans* (Giordani *et al.*, 2004).

To analyse the interaction of drug combinations (THY–FLC and THY–AMB), we used two non-parametric approaches, FICI and ΔE , based on the LA and BI interaction theories, respectively. The interaction between FLC and THY against the *C. albicans* strains is presented in Table 2. There was good agreement between the FICI and ΔE methods for the THY–FLC and THY–AMB combinations in all *C. albicans* strains tested, and we did not find antagonistic interactions between FLC–THY or AMB–THY in these FLC-resistant *C. albicans* and FLC-susceptible *C. albicans*.

For all 25 FLC-resistant *C. albicans* strains tested, the interaction between FLC and THY was synergistic in 24 out of 25 strains by the FICI method, with FICIs ranging from 0.25 to 0.5. By the ΔE method, 22 out of 25 strains showed a high percentage of synergistic interactions, ranging from 109.8 to 696.5 % (Table 2). The interaction between AMB and THY was synergistic in 22 out of 25 strains by the FICI method, with FICIs ranging from 0.25 to 0.5. By the ΔE method, 22 out of 25 strains showed a high percentage of synergistic interactions, ranging from 112.8 to 532.5 % (Table 3).

For 12 clinical isolates of *C. albicans* susceptible to FLC tested, the interaction between FLC and THY was synergistic in 10 out of 12 strains by the FICI method, with FICIs ranging from 0.25 to 0.5. By the ΔE method, 9 out of 12 strains showed high percentages of synergistic interactions, ranging from 119.2 to 299.8 % (Table 2). The interaction between AMB and THY was synergistic in 8 out

Table 1. Susceptibilities of *C. albicans* strains against THY alone and in combination with FLC or AMB

Strain (no.)	Median MIC (range) of drug ($\mu\text{g ml}^{-1}$)							FICI (range)	
	Alone			In combination				FLC + THY	AMB + THY
	THY	FLC	AMB	THY*	THY†	FLC	AMB		
Clinical isolates of <i>C. albicans</i> resistant to FLC (22)	128 (32–256)	128 (≥ 64)	0.5 (0.063–1)	64 (32–128)	64 (32–128)	32 (2–128)	0.25 (0.016–0.5)	0.5 (0.25–1)	0.5 (0.25–1.5)
<i>C. albicans</i> TW 00313 (1)	128	128	1	64	32	64	0.25	1	0.5
<i>C. albicans</i> TW 07239 (1)	128	512	0.5	64	32	8	0.125	0.5	0.5
<i>C. albicans</i> S-1 (1)	256	>512	0.5	64	64	512	0.125	0.5	0.5
<i>C. albicans</i> ATCC 10231 (1)	128	1	0.125	32	64	0.5	0.063	0.75	1
Clinical isolates of <i>C. albicans</i> susceptible to FLC (12)	128 (64–128)	2 (0.25–2)	0.25 (0.125–0.5)	64 (32–64)	64 (32–64)	0.25 (0.03–0.25)	0.03 (0.03–0.06)	0.5 (0.25–1.0)	0.5 (0.5–1)

*MIC of THY indicated when THY in combination with FLC.

†MIC of THY indicated when THY in combination with AMB.

Table 2. *In vitro* interaction between THY and FLC

For the FICI model, synergy was defined as a FICI of ≤ 0.5 , antagonism was defined as a FICI of >4 , and indifference was defined as a FICI of >0.5 to 4 (i.e. no interaction). For the ΔE model, $\sum \text{SYN}$ and $\sum \text{ANT}$ were the sums of the percentages of all statistically significant synergistic and antagonistic interactions. Interactions with $<100\%$ statistically significant interactions were considered weak synergism, those with 100 to 200 % statistically significant interactions were considered moderate, and those with $>200\%$ statistically significant interactions were considered strong.

Strain	Non-parametric method				
	FICI		ΔE model		
	Mean (range)	Interpretation	$\sum \text{SYN}$ (n)	$\sum \text{ANT}$ (n)	Interpretation
Clinical isolates of <i>C. albicans</i> resistant to FLC					
<i>C. albicans</i> 762	0.281 (0.125–0.313)	SYN	185.7 (18)	–30.4 (6)	SYN
<i>C. albicans</i> 876	0.25 (0.14–0.25)	SYN	86.9 (24)	–30.5 (10)	IND
<i>C. albicans</i> 904	0.5 (0.25–0.5)	SYN	121.8 (13)	–11.7 (4)	SYN
<i>C. albicans</i> 885	0.25 (0.145–0.25)	SYN	198.9 (17)	–18.3 (5)	SYN
<i>C. albicans</i> 817	0.375 (0.25–0.375)	SYN	191.7 (18)	–19.2 (5)	SYN
<i>C. albicans</i> 580	0.5 (0.375–0.516)	SYN	113.8 (21)	–28.2 (12)	SYN
<i>C. albicans</i> 502	0.5 (0.25–0.5)	SYN	127.7 (17)	–38.4 (14)	SYN
<i>C. albicans</i> 0612197	0.5 (0.375–0.5)	SYN	121.6 (19)	–42.6 (6)	SYN
<i>C. albicans</i> 958	0.375 (0.25–0.375)	SYN	194 (16)	–5 (2)	SYN
<i>C. albicans</i> 0604109	0.5 (0.25–0.5)	SYN	155.6 (28)	–17.8 (4)	SYN
<i>C. albicans</i> 687	0.375 (0.28–0.5)	SYN	194.8 (5)	–8.8 (3)	SYN
<i>C. albicans</i> 893	0.5 (0.375–0.5)	SYN	30.1 (5)	–80.1 (7)	IND
<i>C. albicans</i> 584	0.5 (0.25–0.5)	SYN	109.8 (15)	–23.5 (5)	SYN
<i>C. albicans</i> 659	0.25 (0.14–0.25)	SYN	696.5 (25)	–65.3 (6)	SYN
<i>C. albicans</i> 0710498	0.28 (0.25–0.28)	SYN	167.3 (12)	–20.8 (4)	SYN
<i>C. albicans</i> 891	0.25 (0.14–0.375)	SYN	135.6 (12)	–22.1 (6)	SYN
<i>C. albicans</i> 754	0.32 (0.28–0.32)	SYN	188.2 (17)	–26.6 (8)	SYN
<i>C. albicans</i> 249	0.5 (0.25–0.5)	SYN	191.1 (18)	–12.2 (6)	SYN
<i>C. albicans</i> 954	0.313 (0.25–0.313)	SYN	195.3 (28)	–17.6 (5)	SYN
<i>C. albicans</i> 349	0.5 (0.25–0.5)	SYN	125.6 (25)	–45.5 (8)	SYN
<i>C. albicans</i> 842	0.5 (0.25–0.5)	SYN	230.5 (21)	–10.2 (5)	SYN
<i>C. albicans</i> 0612194	0.375 (0.25–0.375)	SYN	259.5 (19)	–23.6 (6)	SYN
<i>C. albicans</i> S-1	0.5 (0.25–0.5)	SYN	136.5 (22)	–65.3 (6)	SYN
<i>C. albicans</i> TW 00313	1.0 (0.5–1.0)	IND	56.5 (4)	–88.2 (7)	IND
<i>C. albicans</i> TW 07239	0.5 (0.375–0.5)	SYN	180.8 (19)	–14.6 (5)	SYN
<i>C. albicans</i> ATCC 10231	0.75 (0.5–0.75)	IND	90.5 (14)	–58.5 (14)	IND
Clinical isolates of <i>C. albicans</i> susceptible to FLC					
<i>C. albicans</i> 380	0.375 (0.28–0.5)	SYN	189.8 (8)	–10 (4)	SYN
<i>C. albicans</i> 557	0.5 (0.375–0.5)	SYN	119.2 (10)	–40.1 (9)	SYN
<i>C. albicans</i> 822	0.313 (0.313–0.5)	SYN	192 (36)	–4 (2)	SYN
<i>C. albicans</i> 465	0.28 (0.25–0.32)	SYN	175.2 (13)	–19.6 (4)	SYN
<i>C. albicans</i> 271	0.313 (0.25–0.313)	SYN	226.8 (18)	–22.1 (7)	SYN
<i>C. albicans</i> 494	0.25 (0.14–0.28)	SYN	489 (22)	–81.2 (5)	SYN
<i>C. albicans</i> 782	1.0 (0.515–1.0)	IND	91 (24)	–88.5 (20)	IND
<i>C. albicans</i> 629	0.32 (0.28–0.375)	SYN	176.3 (15)	–25.1 (7)	SYN
<i>C. albicans</i> 786	0.28 (0.25–0.28)	SYN	299.8 (18)	–30.3 (4)	SYN
<i>C. albicans</i> 955	0.375 (0.28–0.375)	SYN	178.8 (5)	–17.8 (3)	SYN
<i>C. albicans</i> 505	0.5 (0.375–0.5)	SYN	48.5 (17)	–10.1 (4)	IND
<i>C. albicans</i> 623	1.0 (0.75–1.0)	IND	60.4 (3)	–89.7 (8)	IND

ANT, Antagonism; IND, indifference; SYN, synergism.

of 12 strains by the FICI method, with FICIs ranging from 0.25 to 0.5. By the ΔE method, 9 out of 12 strains showed a high percentage of synergistic interactions, ranging from 106.2 to 302.5 % (Table 3). By comparison with clinical

isolates of *C. albicans* resistant to FLC, we found that isolates of *C. albicans* susceptible to FLC were less sensitive to the combination of AMB and THY. While, both the clinical isolates of *C. albicans* resistant and susceptible to

Table 3. *In vitro* interaction between THY and AMB

For the FICI model, synergy was defined as a FICI of ≤ 0.5 , antagonism was defined as a FICI of > 4 , and indifference was defined as a FICI of > 0.5 to 4 (i.e. no interaction). For the ΔE model, $\sum \text{SYN}$ and $\sum \text{ANT}$ were the sums of the percentages of all statistically significant synergistic and antagonistic interactions. Interactions with $< 100\%$ statistically significant interactions were considered weak synergism, those with 100 to 200 % statistically significant interactions were considered moderate, and those with $> 200\%$ statistically significant interactions were considered strong.

Strain	Non-parametric method				
	FICI		ΔE model		
	Mean (range)	Interpretation	$\sum \text{SYN}$ (n)	$\sum \text{ANT}$ (n)	Interpretation
Clinical isolates of <i>C. albicans</i> resistant to FLC					
<i>C. albicans</i> 762	0.281 (0.125–0.281)	SYN	301.7 (17)	–25.4 (5)	SYN
<i>C. albicans</i> 876	1.5 (0.75–1.5)	IND	25.5 (15)	–58.5 (13)	IND
<i>C. albicans</i> 904	0.625 (0.375–0.625)	IND	149.8 (18)	–68.2 (12)	SYN
<i>C. albicans</i> 885	1.5 (0.75–1.5)	IND	30.2 (12)	–75.4 (10)	IND
<i>C. albicans</i> 817	0.375 (0.28–0.375)	SYN	219.9 (27)	–19.3 (7)	SYN
<i>C. albicans</i> 580	0.5 (0.28–0.5)	SYN	128.7 (19)	–43.4 (16)	SYN
<i>C. albicans</i> 502	0.5 (0.25–0.5)	SYN	117.9 (10)	–15 (4)	SYN
<i>C. albicans</i> 0612197	0.5 (0.375–0.5)	SYN	119.8 (22)	–34.2 (12)	SYN
<i>C. albicans</i> 958	0.281 (0.125–0.281)	SYN	191.8 (12)	–13.7 (4)	SYN
<i>C. albicans</i> 0604109	0.313 (0.25–0.313)	SYN	176.7 (17)	–18.2 (4)	SYN
<i>C. albicans</i> 687	0.5 (0.313–0.5)	SYN	20.1 (4)	–80.1 (7)	IND
<i>C. albicans</i> 893	0.25 (0.14–0.313)	SYN	118.6 (9)	–14.1 (3)	SYN
<i>C. albicans</i> 584	0.28 (0.25–0.28)	SYN	439.2 (24)	–12.2 (4)	SYN
<i>C. albicans</i> 659	0.313 (0.25–0.313)	SYN	190.3 (29)	–16.6 (4)	SYN
<i>C. albicans</i> 0710498	0.375 (0.28–0.5)	SYN	188.8 (22)	–10.8 (3)	SYN
<i>C. albicans</i> 891	0.251 (0.14–0.313)	SYN	196.2 (15)	–30.5 (6)	SYN
<i>C. albicans</i> 754	0.25 (0.14–0.25)	SYN	172.5 (13)	–20.8 (5)	SYN
<i>C. albicans</i> 249	0.5 (0.25–0.5)	SYN	114.6 (22)	–42.5 (7)	SYN
<i>C. albicans</i> 954	0.25 (0.127–0.25)	SYN	235.4 (29)	–12.6 (4)	SYN
<i>C. albicans</i> 349	0.28 (0.125–0.28)	SYN	532.5 (22)	–52.1 (4)	SYN
<i>C. albicans</i> 842	0.25 (0.125–0.25)	SYN	225.5 (21)	–36.3 (6)	SYN
<i>C. albicans</i> 0612194	0.5 (0.25–0.5)	SYN	112.8 (9)	–42.6 (7)	SYN
<i>C. albicans</i> S-1	0.375 (0.25–0.375)	SYN	180.5 (18)	–20.8 (7)	SYN
<i>C. albicans</i> TW 00313	0.5 (0.25–0.5)	SYN	118.5 (22)	–51.4 (8)	SYN
<i>C. albicans</i> TW 07239	0.5 (0.25–0.5)	SYN	190.1 (17)	–10.2 (5)	SYN
<i>C. albicans</i> ATCC 10231	1 (0.75–1)	IND	55.5 (4)	–92.2 (8)	IND
Clinical isolates of <i>C. albicans</i> susceptible to FLC					
<i>C. albicans</i> 380	1.5 (0.75–1.5)	IND	30.5 (18)	–69.3 (15)	IND
<i>C. albicans</i> 557	0.375 (0.313–0.5)	SYN	209.9 (25)	–20.5 (7)	SYN
<i>C. albicans</i> 822	0.75 (0.625–0.75)	IND	67.2 (8)	–35.8 (15)	IND
<i>C. albicans</i> 465	0.28 (0.25–0.28)	SYN	289.3 (19)	–35.1 (9)	SYN
<i>C. albicans</i> 271	0.25 (0.14–0.25)	SYN	305.2 (20)	–30.1 (8)	SYN
<i>C. albicans</i> 494	0.5 (0.25–0.5)	SYN	111.6 (15)	–32.8 (11)	SYN
<i>C. albicans</i> 782	0.313 (0.28–0.313)	SYN	178.7 (27)	–20.6 (5)	SYN
<i>C. albicans</i> 629	1.0 (0.75–1)	IND	106.2 (18)	–68.5 (10)	SYN
<i>C. albicans</i> 786	0.5 (0.313–0.5)	SYN	312.7 (13)	–70.2 (7)	SYN
<i>C. albicans</i> 955	0.28 (0.25–0.28)	SYN	302.5 (20)	–35.8 (5)	SYN
<i>C. albicans</i> 505	0.5 (0.375–0.5)	SYN	135.9 (27)	–50.6 (9)	SYN
<i>C. albicans</i> 623	0.625 (0.5–0.625)	IND	88.5 (18)	–15.7 (8)	IND

ANT, Antagonism; IND, indifference; SYN, synergism.

FLC have the similar sensitivity to the combination of FLC and THY.

Among the models and approaches described for the study of *in vitro* drug interactions, the LA and BI theories are the

two major referenced models (Greco *et al.*, 1995). The methods used in this study, FICI and ΔE , are non-parametric models based on the LA and BI theories, respectively. In the FICI model, concentrations of the

drugs, alone or in combination, that produce the same effect, are prepared, whereas in the ΔE model, estimates of the combined effects based on the effects of the individual drugs were compared with those obtained from the experiment. The ΔE model, developed in recent years, is a useful tool for analysing the nature of interactions between different drugs based on a chequerboard method. When it was used for assessing the interaction between THY–FLC or AMB–THY, the percentages of fungal growth were derived from the experimental data directly, and good agreement was found with the FICI model (Sun *et al.*, 2008); our results also showed good concordance between the FICI and ΔE models.

From the data above, we can conclude that THY shows potent antifungal effects against clinical *C. albicans* isolates, including FLC-resistant and FLC-susceptible *C. albicans* strains, when combined with FLC or AMB, although it shows weak antifungal activity when used alone.

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