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The antifungal activities and biological consequences of BMVC-12C-P, a carbazole derivative against *Candida* species

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Abstract

Fungal infections, particularly *Candida* species, have increased worldwide and caused high morbidity and mortality rates. The toxicity and development of resistance in present antifungal drugs justify the need of new drugs with different mechanism of action. BMVC-12C-P, a carbazole-type compound, has been found to dysfunction mitochondria. BMVC-12C-P displayed the strongest antifungal activities among all of the BMVC derivatives. The minimal inhibitory concentration (MIC) of BMVC-12C-P against *Candida* species ranged from 1 to 2 μg/ml. Fluconazole-resistant clinical isolates of *Candida* species were highly susceptible to BMVC-12C-P. The potent fungicidal activity of BMVC-12C-P relates to its impairing mitochondrial function. Furthermore, we found that the hyphae growth and biofilm formation were suppressed in *C. albicans* survived from BMVC-12C-P treatment. This study demonstrates the potential of BMVC-12C-P as an antifungal agent for treating *Candida* infections.

Key words: *Candida* species, BMVC-12C-P, mitochondria, fungicidal activity.

Introduction

Invasive candidiasis is a serious problem that often causes approximately 30–45% mortality in critical patients.[1] Azole antifungal agents are the major drugs used for treating fungal infections; however, severe side effects are reported.[2] Meanwhile, the development of azole-resistant isolates of *Candida* species is increasing.[3] In this regard, development of new antifungal drugs with lower cytotoxicity and different mechanism of action is an alternative approach for treating fungal infection.

Carbazole is a nitrogen-containing aromatic compounds. This tricyclic compound consists of two six-membered benzene rings, which was fused to either side of a pyrrole ring.[4] Carbazole-type chemicals synthesized or isolated from natural sources have been reported to have fungistatic or fungicidal activity against *Candida* species.[4,5] However, the mechanism of fungicidal activity of carbazoles is still unclear. 3,6-bis (1-methyl-4-vinylpyridinium) carbazole diiodide (BMVC) is synthesized from 3,6-dibromocarbazole and carries cationic charges.[6] BMVC has been shown to accumulate in the nucleus of mammalian cells, bind to and thermally stabilize DNA.[7] On the other hand, the derivative of BMVC, 3,6-bis (1-methyl-4-vinylpyridinium iodide)-9-(1-(1-methyl-piperidinium iodide) dodecyl) carbazole (BMVC-12C-P) has been found to mainly accumulated in the mitochondria of cancer cells.[8] It has been shown that the accumulation of BMVC-12C-P in mitochondria increased the amounts of reactive oxygen species, resulting in the decrease of mitochondrial membrane potential, cytochrome c release and cell death.[9] BMVC-12C-P has preferential cytotoxicity against cancer cells but not normal mammalian fibroblasts cells.[8,9] Furthermore, we found that BMVC-12C-P could stabilize the G-quadruplex (G4) structure in mitochondrial DNA and further suppressed the expression of ND3 and COX I gene.[9] These findings suggest BMVC and its derivatives have promising potential as a selective cytotoxic agent.

In this study, we examined whether BMVC and its derivatives were effective against *Candida* species and drug-resistant strains from clinical patients. Since BMVC-12C-P can localize in the mitochondria of mammalian cells,[8,9] we hypothesized that BMVC-12C-P might accumulate in the mitochondria of *Candida* species and further interfere its function. The following biological consequences such as hyphae growth and biofilm formation in *C. albicans* treated with BMVC-12C-P were also addressed. Our results indicate the possibility of BMVC-12C-P as an antifungal drug for treating *Candida* infections.

Methods

Preparation of BMVC and derivatives

The synthesis of BMVC and its derivatives have been described previously.[6,8,9] BMVC and its derivatives powder were dissolved in DMSO and then sonicated at 45°C until it was well dissolved. The absorption at $OD_{430\ nm}$ was measured using UV-visible spectrophotometer DU800 (Beckman Coulter). The concentrations of BMVC and derivatives were calculated according to the Beer's Law ($A = \varepsilon bc$). These compounds were prepared as 1 mM stock solution and stored at 4°C before use.[9]

Candida strains

Candida albicans SC5314 (ATCC MYA-2876D), *Candida glabrata* (ATCC 90030), *C. krusei* (ATCC 6258), *Candida tropicalis* (ATCC 750), and fluconazole-resistant clinical isolates of *Candida glabrata* (F2011c057), *Candida glabrata* (F2011s056), *Candida glabrata* (F2011h057), *Candida tropicalis* (F2011ac003), *Candida krusei* (ATCC 6258), *Candida krusei* (F2011aa023), and *Candida krusei* (F2011r076) were generous gifts from Dr. Yee-Chun Chen. The yeast peptone dextrose (YPD, Difco, Detroit, MI, USA), and YPD agar plate were used to maintain the *Candida* species.

Antifungal susceptibility testing

The MIC was determined by the broth dilution antifungal susceptibility test of yeasts described in the protocol of M27-A3 established by the Clinical and Laboratory Standards Institute (CLSI).[10] *Candida* species were diluted in Roswell Park Memorial Institute (RPMI) 1640 medium with 0.165 mol/l 3-(N-morpholino) propanesulfonic acid, MOPS, and 2% glucose, pH7.0 until the concentration was 5×10^2 to 2.5×10^3 cfu/ml and then incubated with the BMVC-12C-P or fluconazole (Sigma-Aldrich, St. Louis, MO, USA) for 48 hours at 37°C. Drug concentrations were 0.125–64 μg/ml. The MIC_{50} and MIC_{100} was defined as the concentration of drug at which 50% and 100% of growth was inhibited, respectively.

The minimal fungicidal concentration (MFC) followed the MIC experiment, and the sample was spread on YPD agar plates at 37°C for 18 hours. The MFC was defined as the lowest drug concentration that colony amount was three or fewer that indicated 99 % of the inoculum was killed.

Subcellular location of BMVC-12C-P and Mitochondrial architecture

In total, 10^7 cfu/ml *C. albicans* were incubated with BMVC-12C-P for 24 hours. Before observation, the *C. albicans* were separately stained with Hoechst 33342 and Mitotracker red for 30 minutes. Images were captured by Leica SP2 confocal scanning fluorescence microscope (Leica Inc., Malvern, PA, USA). The excitation wavelength of the BMVC-12C-P was 458 nm, and the absorption wavelength was 500–600 nm; the Hoechst 33342 was 405 nm and the absorption wavelength was 450–500 nm; the Mitotracker red was 543 nm and the absorption wavelength was 600–700 nm.

To examine the mitochondrial architecture of *C. albicans* treated with BMVC-12C-P, 10^7 cfu/ml cells were treated with 21 μg/ml (about 20 μM) BMVC-12C-P for 30 minutes in phosphate-buffered saline (PBS; pH 7.4) at 37°C. After that, cells were stained with mitotracker red CMXRos for 30 minutes in PBS at 37°C and then washed three times by PBS. Images were captured by Leica SP2 confocal scanning fluorescence microscope (Leica Inc., Malvern, PA, USA). The excitation wavelength of the CMXRos was 579 nm, and the absorption wavelength was 599 nm.

Antifungal cytotoxicity assays

To determine the antifungal activity in planktonic cells, 10^7 cfu/ml of *C. albicans* were collected and washed three times by PBS. Cells were incubated with BMVC or its derivatives in PBS at 37°C for 24 hours with shacked at 200 rpm. The concentration of BMVC or its derivatives used in each experiment was indicated in figure legends. To determine the cellular viability, cells were serially diluted with PBS from 10^{-1} to 10^{-5} times and plating on YPD agar and incubated at 37°C for 18 hours. The number of colonies were counted by averaging the cfu from at least three independent experiments.

Mitochondrial function analysis

To examine the effect of BMVC-12C-P on mitochondria, *C. albicans* were grown in yeast extract-peptone containing either fermentable carbon source (2% dextrose) named as YPD or non-fermentable carbon source (2% glycerol) named as YPG. Briefly, cells (5×10^2 to 2.5×10^3 cfu/ml) were treated with 1, 2, 4, 8, and 16 μg/ml BMVC-12C-P in YPD or YPG for 24 hours at 37°C. Then, the samples were serially diluted 10-fold with PBS from 10^{-1} to 10^{-5} times and plated on YPD agar. The number of viable cells were counted and expressed as cfu.

Oxygen consumption was assessed using the Seahorse XF24 extracellular flux analyzer (Seahorse Bioscience, USA). Briefly,

10^3 cells/ml of *C. albicans* cultures were treated with 1, 2, and 4 μg/ml BMVC-12C-P for 24 hours at 37°C in YPG. The measurement of mitochondrial oxygen consumption rate (OCR) was performed according to the manufacturer's instructions.

To deplete mtDNAs in *C. albicans*, the wild-type cells were grown to saturation for two cycles in YPD containing 25 and 50 μg/ml ethidium bromide (Sigma, St. Louis, MO, USA) as conventionally designated *rho*$^-$ cells. The mt*COX1* and mt*NAD3* analyzed by quantitative polymerase chain reaction (Q-PCR) were used to estimate the mitochondrial number. To measure the antifungal activity in *rho*$^-$ cells, antifungal cytotoxicity assay as described above was performed.

Quantitative PCR

The DNA of *C. albicans* treated with or without BMVC-12C-P or EtBr was extracted by NautiaZ Bacteria/Fungi DNA Kit (Nautiagene). Q-PCR analysis using the Fast SYBR® Green Master Mix (Applied Biosystems, USA) with the ABI StepOne system (Thermo Fisher Scientific) was performed to analyze the nuclear (qPCR-18S rRNA-F/R) and mtDNA (qPCR-*COX1* and *NAD1*-F/R). The primer sequences were as follows: 18S rRNA-F, 5′-CGCAAGGCTGAAACTTAAAGG-3′;18S rRNA-R, 5′-AGCAGACAAATCACTCCACC-3′; *COX1*-F, 5′-GGTGAATTACGTCTAGCTGTTCC-3′; *COX1*-R, 5′-GCA CCATCTAATAGCCCTACTCA-3′; *NAD3*-F, 5′-TAGGTTG TGTTGCTGAATGTGC-3′; *NAD3*-R, 5′-CCAGTACCACC ACCCATAAATAAG-3′.

Cell death mode analysis

In summary, 10^7 cfu/ml *C. albicans* were incubated with 42 μg/ml BMVC-12C-P in PBS for 0.5 hours at 37°C. After incubation, cells were washed with PBS and stained with the mixture of 0.5 μM DAPI and 0.5 μM propidium iodide (PI) for 20 minutes, then washed with PBS. The cells were then harvested and examined by the Eclipse Ti inverted microscope (Nikon Instruments Inc., Melville, NY, USA).

Morphologic analysis

C. albicans (10^7 cfu/ml) was washed three times by PBS then incubated with 5.25, 7.875, 10.5, 21, and 42 μg/ml BMVC-12C-P for 24 hours in RPMI 1640-MOPS at 37°C and shacked at 200 rpm. The cell suspension was observed for the hyphal formation by an Eclipse Ti inverted microscope (Nikon Instruments Inc., Melville, NY, USA).

Biofilm and biomass analysis

For the biofilm study, *C. albicans* were cultured using silicone model to measure the dry weight of biofilms.[11] Briefly, preweighted sterile silicone squares (1.5 cm × 1.5 cm, Cardiovascular Instruments Corp., PR72034-06N) were preincubated in fetal bovine serum (FBS) for 16 hours at 37°C with 125 rpm shaking in a 12-well plastic plate. After removing FBS, silicone squares were washed with PBS and added 2×10^7 *C. albicans* in 2 ml RPMI 1640-MOPS medium at 37°C for 90 minutes with shacked at 125 rpm. The silicone squares were washed three times with PBS and then placed in new 12-well plastic plate. The silicone squares were incubated in 2 ml RPMI 1640-MOPS medium containing 5.25, 10.5, and 21 μg/ml BMVC-12C-P at 37°C for 60 hours under 125 rpm shacked. After removing the supernatants, the silicone squares were dry for 2 days at room temperature, then weighing to determine the biofilm mass.

Statistical analysis

Each value represented the mean ± SD from three independent experiments. Comparisons between two groups were analyzed by two-tailed Student *t* test. A value of $P < .05$ was considered statistically significant.

Results

Antifungal activity of BMVC and its derivatives

To examine the antifungal activity of BMVC and its derivatives (Fig. 1a), *C. albicans* was incubated with BMVC and its derivatives for 24 hours. Compared to the control group, no colony was found in *C. albicans* treated with 63 μg/ml of BMVC-12C-P, indicating a complete cell killing (Fig. 1b). Meanwhile, there is only a 3-log reduction of cfu in *C. albicans* treated with 63 μg/ml of BMVC-9C-P. However, no significant toxicity was found in cells treated with BMVC, BMVC-4C-P or BMVC-8C-P. The cytotoxicity of BMVC-12C-P against *C. albicans* was also shown in a dose-dependent (Fig. 1c) and time-dependent manner with a complete killing after 12 hours of incubation (Fig. 1d). Taken together, these results indicate that BMVC-12C-P has antifungal ability against *C. albicans*.

The antifungal spectrum of BMVC-12C-P

We then further addressed the potential of BMVC-12C-P as an antifungal drug by examining the *in vitro* activity against the *Candida* species strains and fluconazole-resistant strains of *Candida* species. As shown in Table 1, the MIC_{50} of fluconazole against *C. albicans* (SC5314), *C. glabrata* (ATCC 90030), *C. krusei* (ATCC 6258), and *C. tropicalis* (ATCC 750) were 0.125, 4, 16, and 1 μg/ml, respectively. In contrast, MIC_{50} of BMVC-12C-P were 0.5, 2, 2, and 0.5 μg/ml, respectively. Except for the *C. albicans* (SC5314), the MIC_{50} of BMVC-12C-P against the *Candida* species is lower than that of fluconazole. Most importantly, MIC_{50} of BMVC-12C-P against fluconazole-resistant isolates of *Candida* species ranged from 0.5 to 4 μg/ml, which were about 16-fold lower than fluconazole (higher than 64 μg/ml).

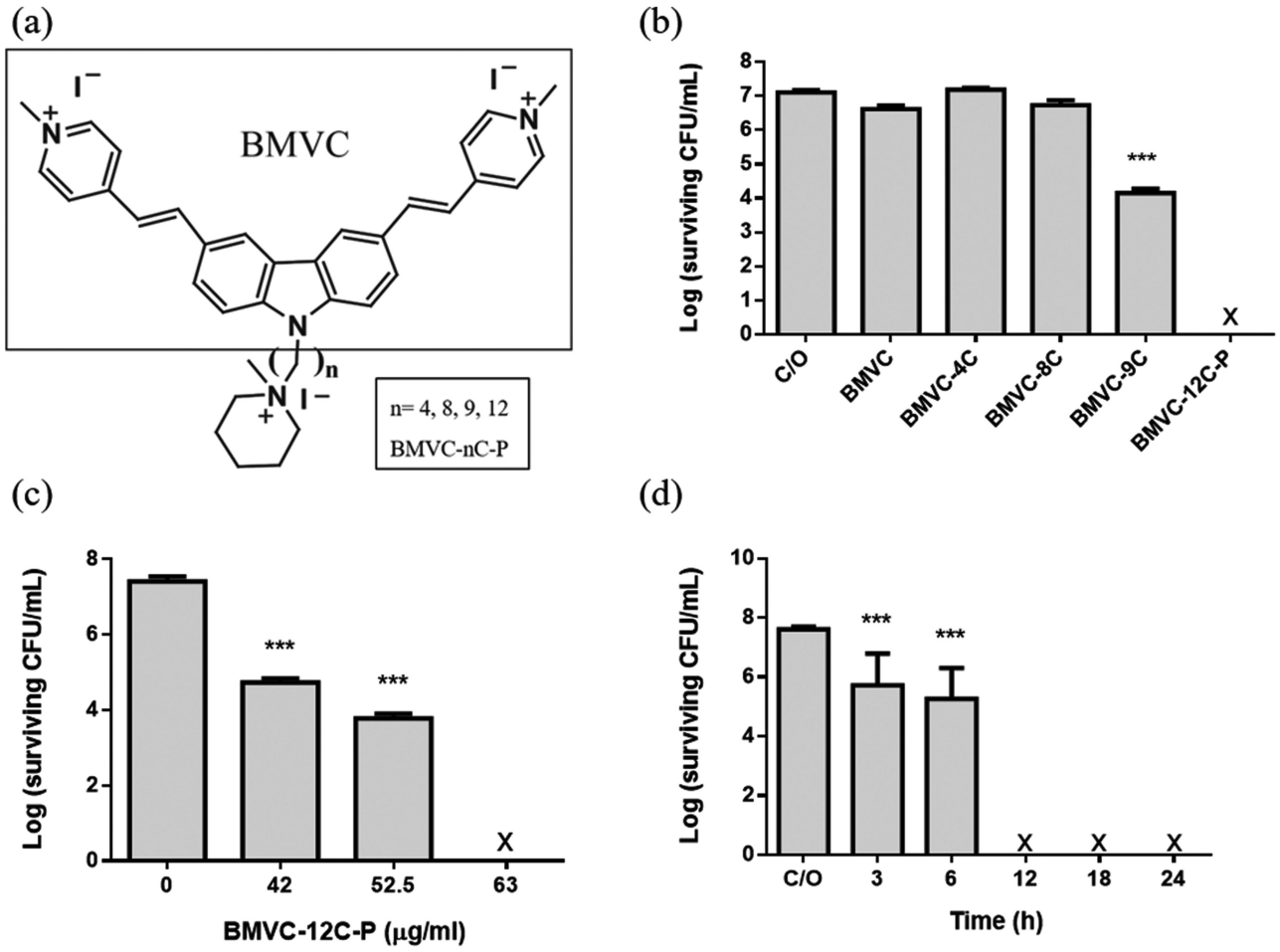

Figure 1. The surviving of *C. albicans* treated with BMVC and its derivatives. (a) Chemical structure of BMVC and its derivatives. (b) *C. albicans* suspension containing 10^7 CFU/mL was incubated with 63 μg/ml BMVC and its derivatives for 24 h, then subjected to a plate count for measuring cell viability. *C. albicans* were incubated with 42, 52.5, and 63 μg/ml BMVC-12C-P for 24 h (c) and 63 μg/ml BMVC-12C-P for 3 to 24 h (d). Cell surviving was determined by plate count. At least 3 repeated experiments were performed to determine the surviving CFU/mL. X indicates the complete eradication of cells. ***$p < 0.001$ as compared to control.

Table 1. The MICs (μg/ml) and MFC (μg/ml) of BMVC-12C-P and fluconazole against *Candida* species strains and fluconazole-resistant clinical isolates of *Candida* species.

	Fluconazole		BMVC-12C-P			
Species	MIC_{50}	MFC	MIC_{50}	MIC_{100}	MFC	MFC/MIC
Candida albicans (SC5314)	0.125	>64	0.5	2	2	1
Candida glabrata (ATCC 90030)	4	>64	2	4	4	1
Candida krusei (ATCC 6258)	16	64	2	4	4	1
Candida tropicalis (ATCC 750)	1	16	0.5	2	2	1
Candida glabrata (F2011h057)	>64	>64	4	8	8	1
Candida glabrata (F2011c057)	>64	>64	4	4	4	1
Candida glabrata (F2011s056)	>64	>64	4	8	8	1
Candida krusei (F2011aa023)	64	>64	4	4	4	1
Candida krusei (F2011r076)	>64	>64	2	4	4	1
Candida tropicalis (F2011ac003)	>64	>64	0.5	2	2	1

In fact, the MIC_{100} of BMVC-12C-P against *Candida* species and drug-resistant clinical isolates ranged from 2 to 8 μg/ml. As the MFC/MIC of BMVC-12C-P is around 1, indicating its action is fungicidal.[12,13] These results revealed that BMVC-12C-P exhibited a broad-spectrum and potent activity against a variety of *Candida* species.

BMVC-12C-P disrupts mitochondrial function of *C. albicans*

Previously, BMVC-12C-P has been shown to mainly localize at the mitochondria of cancer cells.[9] Therefore, we further examined whether BMVC-12C-P was also localized at the mitochondria of eukaryotic microbial cells. The cellular localization in *C. albicans* was observed under confocal microscope. For comparison, Hoechst 33342 and MitoTracker red was used to co-localize the nucleus and mitochondria, respectively. As shown in Fig. 2a, BMVC-12C-P was mainly localized at mitochondria as revealed by the superimposing of BMVC-12C-P and MitoTracker red. Further study showed that mitochondrial architecture of *C. albicans* treated with BMVC-12C-P became swollen and fragmented (Fig. S1).

To elucidate whether BMVC-12C-P impacts the mitochondrial function of *C. albicans*, cells were cultured in the YPG medium containing 2% glycerol as nonfermentable carbon source in which mitochondrial respiration is required for yeast growth. We argued that the growth of *C. albicans* will be significantly reduced in YPG medium if the mitochondrial respiratory function was affected by BMVC-12C-P. Fig. 2b showed that *C. albicans* treated with 4 μg/ml of BMVC-12C-P have a 3-log reduction of cfu and were eliminated completely at the concentration of 8 μg/ml in YPG medium. However, the growth of *C. albicans* was not affected even when treated with 16 μg/ml BMVC-12C-P in YPD medium in which the fermentable carbon source could be used to produce ATP for the growth of *C. albicans*. The dramatic contrast of grown sensitivity between YPD and YPG medium indicates that BMVC-12C-P impacts the mitochondrial function of *C. albicans*. In addition, we observed that the colony size of *C albicans* treated with BMVC-12C-P became smaller after plating on YPD agar plate (Fig. 2c). The appearance of these tiny colonies has been reported as petite mutations because of the aerobic respiratory chain pathway was block and cannot generate ATP.[14] We then directly examine the effect of BMVC-12C-P on cellular energetics by measuring oxygen consumption rate, which is an indicator of mitochondrial respiration. As shown in Fig. 2d, the consumption of oxygen in *C albicans* decreased dramatically in a dose-dependent manner compared to cells not exposed to BMVC-12C-P. Taken together, these results clearly indicate that the oxidative respiration was inhibited in *C. albicans* treated with BMVC-12C-P, indicating mitochondria might be the major target of BMVC-12C-P.

To further verify the role of mitochondria in the fungicidal activity of BMVC-12C-P, *C albicans* was treated with 25 and 50 μg/ml ethidium bromide (EtBr) to generate respiration-deficient mutants (*rho*$^-$ cells). The *rho*$^-$ cells showed different levels of reduction in mitochondria content as judged by the decrease of two mitochondria DNA (mtDNA), mitochondria-encoded cytochrome c oxidase I (*COX1*) and mitochondria-encoded NADH dehydrogenase 1 (*NAD1*) (Fig. 2e). Cell viability was then compared between the wild-type and different *rho*$^-$ cells in the presence and absence of BMVC-12C-P. As shown in Fig. 2f, the cytotoxic effects of BMVC-12C-P in wild-type cells showed a dose-dependent manner. However, no significant increase in cytotoxicity was found in the *rho*$^-$ cells treated with different concentrations of BMVC-12C-P, indicating its fungicidal activity against *Candida* acts through mitochondria. Previously, BMVC-12C-P was shown to suppress the expression of mitochondrial ND3 and COX I gene in mammalian cells[9]. In this study, we also found the expression of *COX1* gene decreased by 50% in *C. albicans* treated with BMVC-12C-P (Fig. S3), suggesting its antimicrobial activity might relate to its ability to suppress mtDNA replication. To further elucidate the possible cell death mode, *C. albicans* treated with BMVC-12C-P were stained with DAPI and PI. Chromatin condensation was found in cells exposed to BMVC-12C-P, suggesting apoptotic cell death might be induced (Fig. S2).

Effects of BMVC-12C-P on morphogenesis in *C. albicans*

Except for the respiration, mitochondrial function of *Candida* species plays an important role for their pathogenic virulence.[15] Morphological switching and biofilm formation are two of the important virulence factors in *C. albicans*.[16] It has been shown that low concentrations of methylene blue[17] or surfactant[18] could perturb mitochondrial function and repress yeast to hyphal switching in *C. albicans*. We therefore examined weather BMVC-12C-P will affect the hyphal growth in RPMI medium. As shown in Fig. 3a, the hyphal formation was significantly reduced in the presence of 7.875 μg/ml BMVC-12C-P and a higher concentration (10.5 μg/ml) of BMVC-12C-P can completely inhibit the filamentation.

The yeast-to-hypha transition is important for biofilm formation, which is another morphogenetic type of *C. albicans*. As BMVC-12C-P could suppress the hyphae growth, we therefore further examined its effect on the biofilm formation by using silicone model.[11] As shown in Fig. 3b, while the average mass of biofilm formed by *C. albicans* was about 25 mg, a reduced biofilm biomass was found in cells treated with BMVC-12C-P, showing a dose-dependent manner. These results indicate that mitochondrial dysfunction induced by BMVC-12C-P not only results in the suppression of hyphal growth but also biofilm formation in *C. albicans*.

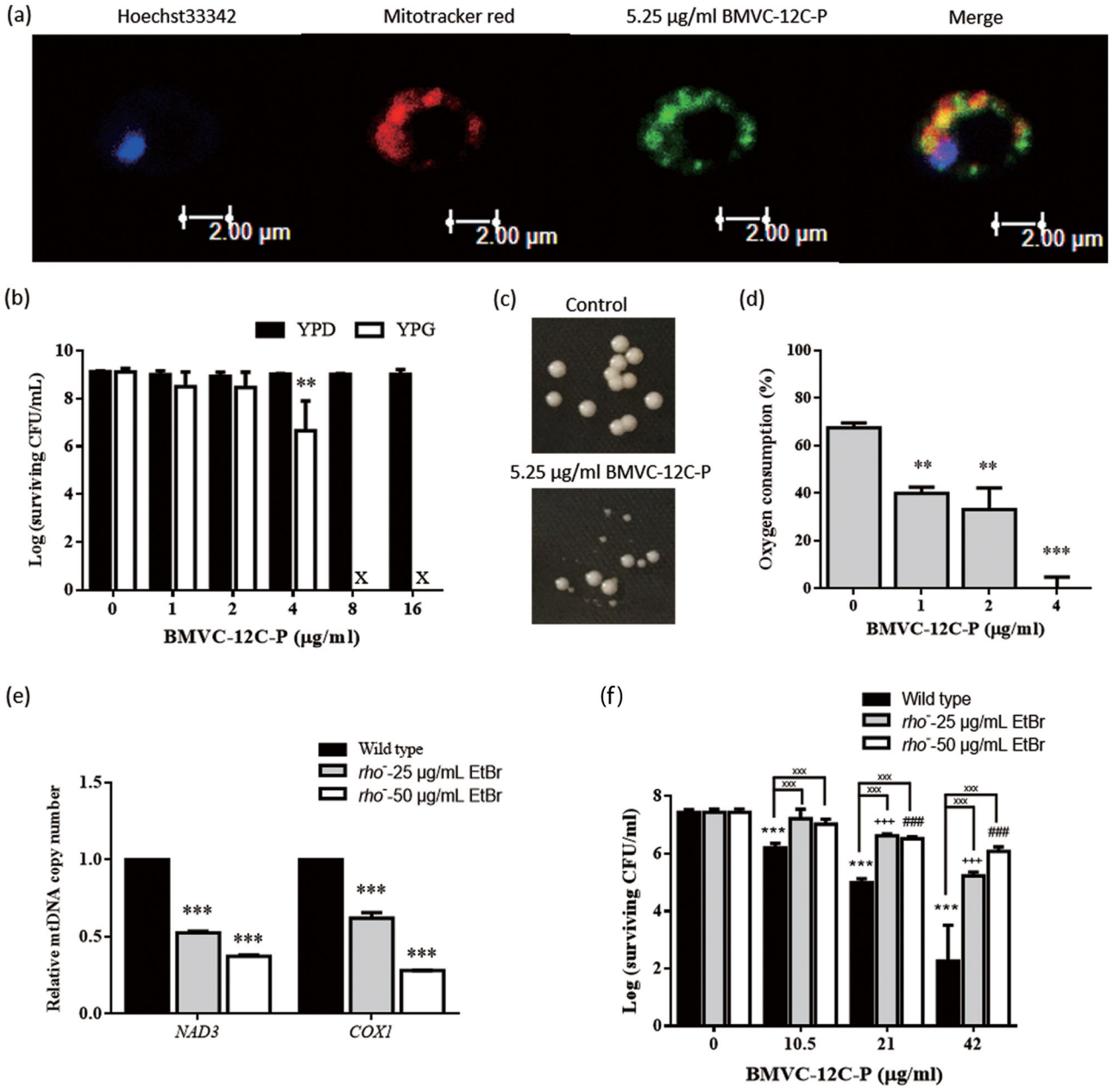

Figure 2. Effect of BMVC-12C-P on the mitochondrial function of *C. albicans*. (a) Cells were incubated with 5.25 μg/ml BMVC-12C-P for 24 h, and then stained with Mitochondria tracker red and Hoechst33342. The images were taken by confocal microscopy to visualize the intracellular localization of BMVC-12C-P as well as the mitochondria and nucleus. (b) Cells were cultured in YPG or YPD media containing different concentrations of BMVC-12C-P for 24 h. Then, cell suspensions were diluted and spread onto YPD agar plates for counting surviving colonies. ** $p < 0.01$ as compared to control in YPG. (c) The petite colonies on the YPD agar plate were obtained from *C. albicans* treated with 5.25 μg/ml BMVC-12C-P for 24 h. (d) For oxygen consumption study, *C. albicans* were cultured in YPG containing 0 to 4 μg/ml BMVC-12C-P for 24 h. ** $p < 0.01$, *** $p < 0.001$ as compared to control. (e) To obtain the *rho-* cells, *C. albicans* were grown in medium containing EtBr as described in Materials and methods. The *COX1* and *NAD3* (mitochondrial DNA) and 18S rRNA (nuclear DNA) in wild type cells and *rho-* cells were measured by Q-PCR. *** $p < 0.001$ as compared to wild type. (f) To perform antifungal assay, wild-type and *rho-* cells were treated with 10.5, 21 and 42 μg/ml of BMVC-12C-P for 24 h and then subjected to plate count. X indicates the complete eradication of cells. *** $p < 0.001$ as compared to wild type without BMVC-12C-P treatment; $^{+++}$ $p < 0.001$ as compared to *rho-* cells (25 μg/ml EtBr treatment) without BMVC-12C-P treatment, $^{\#\#\#}$ $p < 0.001$ as compared to *rho-* cells (50 μg/ml EtBr treatment) without BMVC-12C-P treatment, xxx $P < 0.001$ as compared to wild type under the same conceraction of BMVC-12C-P treatment.

Discussion

Carbazole-type chemicals have been found to induce mitochondrial dysfunction and result in apoptosis of cancer cells.[19] In addition, the antimicrobial activity of carbazole derivative against bacteria and fungi was also reported.[20] The assessment of BMCV-12C-P action was first addressed in the mitochondria of cancer cells.[9] Correlated with mitochondrial dysfunction, growth inhibition and apoptosis were found in

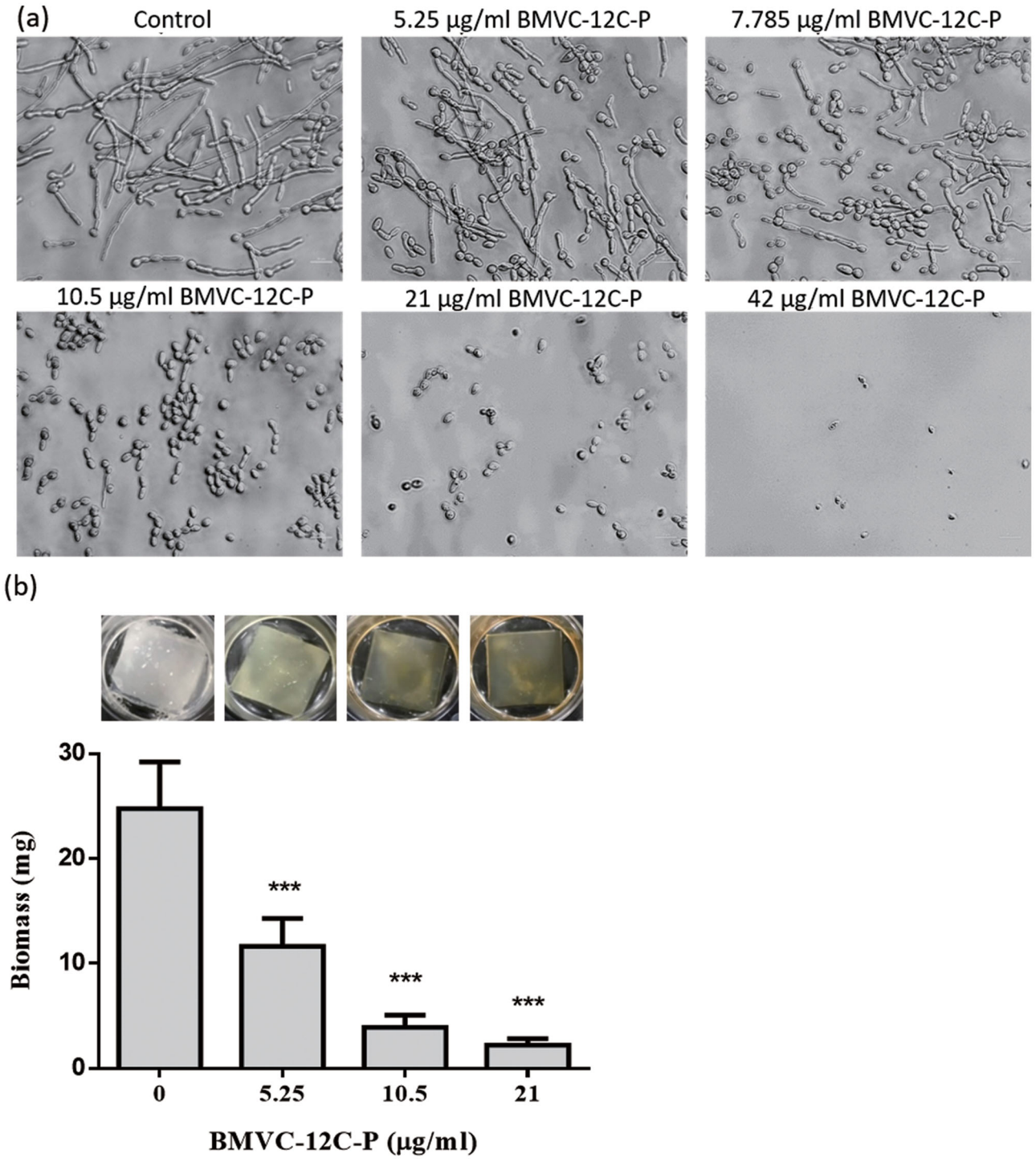

Figure 3. Effects of BMVC-12C-P on hyphae growth and biofilm formation of *C. albicans*. (a) Cells were incubated with different concentrations of BMVC-12C-P for 24 h, then the hyphal formation was observed under bright-field microscopy and photographed. Scale bar = 10 μm. (b) For biofilm formation, cells were inoculated on silicone squares with different concentrations of BMVC-12C-P for 60 h, then photographed (upper panel). After removing the supernatant and dry, the biofilm mass was determined (lower panel). ***$P < 0.001$ for the difference with the control without BMVC-12C-P treatment.

cancer cells treated with BMVC-12C-P. In this study, the MIC_{100} of BMVC-12C-P was ranged from 2 to 8 μg/ml against *Candida* species and drug-resistant clinical isolates. However, there was no appreciable cytotoxic effects on normal mammalian fibroblasts under 10 μM (about 10.5 μg/ml) of BMVC-12C-P treatment.[9] These results indicate that the antifungal toxicity of BMVC-12C-P is significantly higher than its toxicity to the mammalian cells.

In this study, we demonstrated that BMVC-12C-P had a broad spectrum of fungicidal activity against *Candida* species as well as the drug-resistant clinical isolates. The decreased surviving ratio in YPG medium, reduced oxygen consumption and reduced cytotoxicity of *rho*$^{-}$ cells indicated that the fungicidal activity of BMVC-12C-P relates to the mitochondrial dysfunction in *C. albicans*. Previously, it has been shown that the lipophilicity of BMVC derivative governs its preferential localization to the mitochondria over the nucleus.[8] Among the BMVC derivatives, BMVC-4C-P and BMVC-8C-P with relatively low lipophilicities localized preferentially to the nuclei, which showed no antifungal activity against *C. albicans* (Fig. 1b). In contrast, BMVC-9C-P and BMVC-12C-P with relative high lipophilicities showed their preferential localization at mitochondria and significant antifungal activity (Fig. 1b). Correlated with the higher cytotoxicity, BMVC-12C-P has higher mitochondria overlay ratio[8] compared to BMVC-9C-P (Fig. 1b), suggesting the fungicidal activity of BMVC derivative relates to its relative amounts at mitochondria. In this study, we also found out BMVC-12C-P could suppress the expression of mitochondrial *COX I* gene in *C. albicans*. In addition to the fungicidal effect, we also found the bactericidal activity of BMVC-12C-P against *Staphylococcus aureus* (data not shown here).

The mitochondrial activity of *C. albicans* is essential in many virulence factors[15] such as cell wall integrity maintained to resist antifungal drug,[21,22] oxidative stress responses for survival[23] and the ability of morphogenesis switch.[17,23–25] In this study, we demonstrated that mitochondrial dysfunction induced by BMVC-12C-P results in suppressing hyphae growth and biofilm formation which are important virulence factors of *C. albicans* to infect humans.[16] Presently, the underlying mechanisms of BMVC-12C-P involved in microbial killing, suppressing hyphae and biofilm formation are under investigation.

In this study, we demonstrated that BMVC-12C-P had a broad spectrum of fungicidal activity against *Candida* species as well as the fluconazole-resistant clinical isolates. The action mechanism of BMVC-12C-P relates to mitochondrial dysfunction and is different from that of fluconazole. Finally, we demonstrated that hyphae growth and biofilm formation was suppressed in cells survived from BMVC-12C-P treatment. In conclusion, the three preferred characteristics of being fungicidal, dysfunction mitochondria and low toxicity to normal mammalian cells make BMVC-12C-P a promising antifungal drug.

Supplementary material

Supplementary data are available at *MMYCOL* online.

Acknowledgments

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Author contributions

M.S. participated in designing the experiments, carried out the work and manuscript preparation. P.-T.L. participated in performing the experiment of oxygen consumption and helping manuscript preparation. Y.-J.W. and E.T. participated in analyzing the cytotoxicity of BMVC-12C-P against *Candida* species. C.-H.L. participated in the design of the biofilm experiment. T.-C.C. participated in designing the BMVC and its derivatives. All the authors read and approved the final manuscript. C.-T.C. conceived the study, participated in its design and coordination and finalized the draft of the manuscript.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and the writing of the paper.

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