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Identification of a *Candida albicans* Biofilm Inhibitor

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Abstract

Candida albicans proliferates in the skin and oral cavity and is the causative agent of candida dermatitis and oral candidiasis. *C. albicans* is known to form biofilms on oral mucosa and denture surfaces. Formation of biofilms deteriorates the permeability of antifungal drugs, decreasing their effectiveness. Therefore, in this study, I identified a compound with inhibitory activity against *C. albicans* biofilm formation. Heat shock protein 90 was selected as the target protein, and a potential ligand for the same was extracted and identified as 2-(4-methylpiperazin-1-yl)cyclopentanol. *C. albicans* was then cultured with varying concentrations of this compound: 0 mmol/L, 0.63 mmol/l. 2.5 mmol/l, and 10 mmol/l, and biofilm formation was measured via crystal violet assay. The findings demonstrated that 2-(4-methylpiperazin-1-yl)cyclopentanol substantially inhibits biofilm formation when added at a concentration of 0.63 mmol/l or higher. It is suggested that *C. albicans* could be eliminated more efficiently using this compound in combination with the existing antifungal drug miconazole. Further, the compound may also be useful as a disinfectant for medical devices, such as catheters, to prevent the formation of *C. albicans* biofilms.

Keywords

Biofilm, *Candida albicans*, Antifungal Agent

1. Introduction

Candida albicans is a symbiotic fungus that normally inhabits the skin and gastrointestinal tract, and it is one of the causative fungi of endogenous infections such as mucocutaneous and oral candidiasis [1] [2] [3]. Candidiasis is also known as an acquired immunodeficiency syndrome indicator disease because types such as airway candidiasis are frequently found in human immunodeficiency virus-infected patients [4] [5]. *C. albicans* is known to take two forms:

yeast and hyphal [6] [7]. Previous studies have reported that the hyphae are involved in colonization and biofilm formation [8] [9]. Biofilms are membrane-like structures composed of microorganisms, polysaccharides, extracellular DNA, lipids, and proteins; they physically block the penetration of antibiotics, making it difficult to treat the infections with drugs [10] [11]. *C. albicans* is known to easily form biofilms on biological and abiotic surfaces [12] [13]. Oral candidiasis is intractable due to the formation of biofilms in the oral cavity [14]. In addition, biofilm formation on abiotic surfaces, such as catheter lumens and artificial joints, can lead to prolonged infection [15] [16].

Antifungal agents, such as miconazole, are used to treat candidiasis. However, current antifungal drugs, such as miconazole, do not inhibit biofilm formation. Therefore, in this study, I aimed to identify compounds with an inhibitory effect on *C. albicans* biofilm formation and investigated whether the treatment efficacy could be improved by combining the use of this compound as an adjunct therapy with miconazole.

2. Materials and Methods

2.1. Reagent

2-(4-methylpiperazin-1-yl)cyclopentanol was purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Strains and Culture Conditions

C. albicans ATCC 10231 was purchased from American Type Culture Collection (ATCC, Virginia, USA). *C. albicans* ATCC 10231 was grown for 24 h at 37˚C under aerobic conditions on soyabean-casein digest (SCD) agar medium. The fungus was harvested in SCD broth containing Tween 20 (5% v/v), and miconazole (0 μmol/l, 7.5 μmol/l, 15 μmol/l, 30 μmol/l, 60 μmol/l, and 120 μmol/l) with or without 10 mmol/l 2-(4-methylpiperazin-1-yl)cyclopentanol, and then adjusted each suspension to a 0.01 optical density at 600 nm to produce an inoculum density of 1×10^5 (colony-forming units (CFU)/ml). The obtained fungal suspensions were dispensed onto 96-well polystyrene plates at 200 μl/well and cultured for 48 h at 37˚C under aerobic conditions.

2.3. Molecular Docking

In-silico screening was performed by the docking system SeeSAR 10 (BioSolvelT, Nordrhein-Westfalen, Germany). *C. albicans* heat shock protein 90 (HSP90) nucleotide binding domain was selected as the target protein (Protein Data Bank Code: 6CJJ) for docking simulation. For the compound binding site, the ADP binding site of *C. albicans* HSP90 was used. A database (1,000,000 compounds) owned by Namiki Shoji (Tokyo, Japan) was used as the compound database.

2.4. Crystal Violet Assays

As described above, *C. albicans* ATCC 10231 was cultured on SCD agar. The fungus was harvested in SCD broth alone, and SCD broth containing varying

amounts of 2-(4-methylpiperazin-1-yl)cyclopentanol (0 mmol/l, 0.63 mmol/l, 2.5 mmol/l, and 10 mmol/l), and then adjusted each suspension to a 0.1 optical density at 600 nm to produce an inoculum density of 1×10^6 (colony-forming units (CFU)/ml). The obtained fungal suspensions were dispensed onto 96-well polystyrene plates at 200 µl/well and cultured for 24 h at 37˚C under aerobic conditions for use in the crystal violet assay. The cultures were removed, and each well was washed twice with Milli-Q water. A crystal violet aqueous solution (0.1% w/v) was added to each well (200 µl/well), and the mixture was allowed to stand at ambient temperature (approximately 25˚C) for 20 min. After staining, the crystal violet aqueous solution was removed, and each well was washed twice with Milli-Q water. Ethanol was added to each well (200 µl/well), and the mixture was allowed to stand at ambient temperature (approximately 25˚C) for 20 min. The optical density was measured at 570 nm on a SpectraMax190 Microplate Reader (Molecular Devices Co., Ltd., Tokyo, Japan) to detect the amount of biofilm in the 96-well plate (Thermo scientific, Waltham, MA, USA).

2.5. Statistical Analysis

The results are presented as the mean ± standard deviation (SD). The data were analyzed using the statistical program SigmaPlot 14 (Systat software Inc., Berkshire, UK), and *P* values less than 0.05 were considered to denote statistical significance. Significance for differences between groups was examined using Dunnett's test.

3. Results and Discussions

I carried out an *in-silico* search for compounds that may inhibit biofilm formation using the docking system SeeSAR 10 (BioSolvelT, Nordrhein-Westfalen, Germany). HSP90 of *C. albicans*, which is known to be involved in biofilm formation [17], was selected as the target protein. I attempted to extract a compound that could be a ligand for the protein and identified 2-(4-methylpiperazin-1-yl)cyclopentanol (**Figure 1**).

C. albicans ATCC 10231 was cultured in SCD broth containing differing amounts of the compound: 0 mmol/l, 0.63 mmol/l, 2.5 mmol/l, and 10 mmol/l. The amount of biofilm formed was then quantified via a crystal violet assay. When 2-(4-methylpiperazin-1-yl)cyclopentanol was added at a concentration of 0.63 mmol/l or higher, the amount of biofilm formed was significantly lower than that in the control group (**Figure 2**).

These results indicate that 2-(4-methylpiperazin-1-yl)cyclopentanol inhibits biofilm formation by *C. albicans*. Furthermore, the antifungal effects of miconazole alone and miconazole in combination with this compound were compared. When miconazole was used with 2-(4-methylpiperazin-1-yl)cyclopentanol, the effective concentration of miconazole resulting in 50% reduction of *C. albicans* proliferation (EC_{50}) was 7.5 µmol/l or less, whereas when miconazole was used alone, EC_{50} was 60 µmol/l (**Figure 3**).

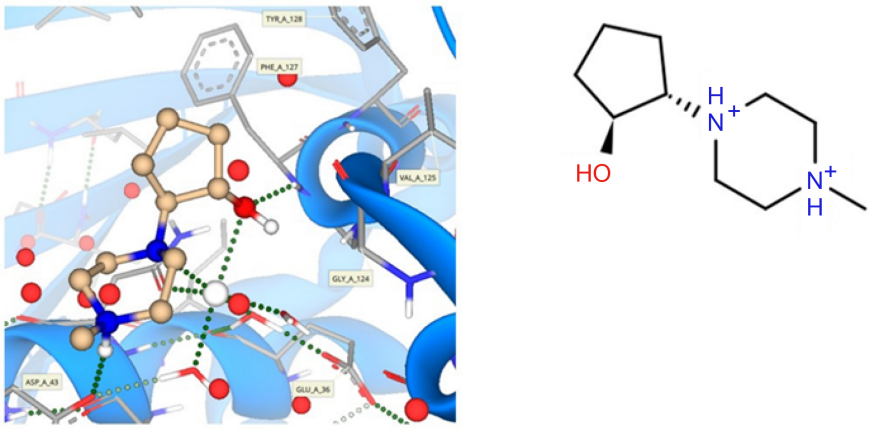

Figure 1. (a) Docking of 2-(4-methylpiperazin-1-yl)cyclopentanol to *Candida albicans* HSP90. (b) Structure of 2-(4-methylpiperazin-1-yl)cyclopentanol.

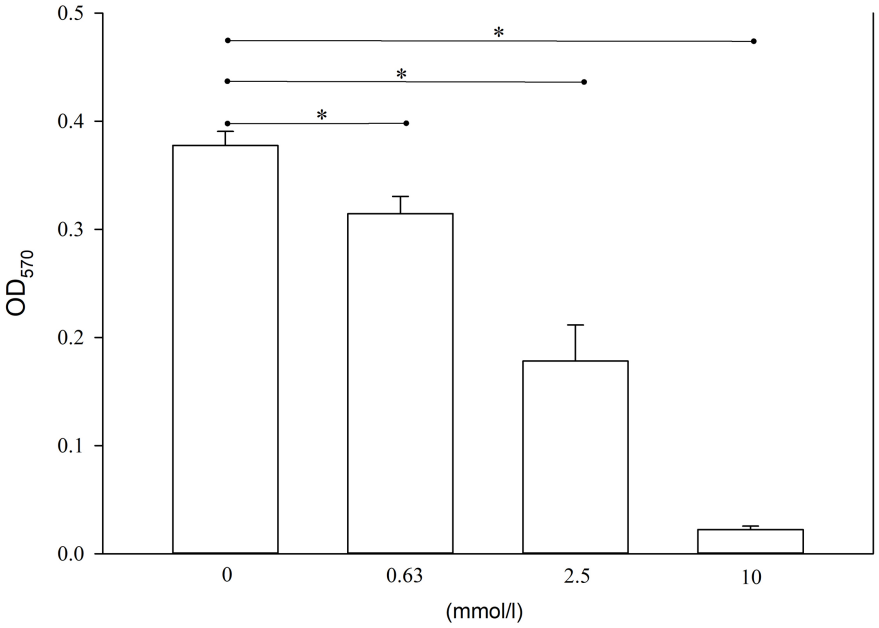

Figure 2. Biofilm formation inhibitory effect of 2-(4-methylpiperazin-1-yl)cyclopentanol. This figure shows results of the crystal violet assay. The data are presented as the mean ± SD (n = 3). Asterisks indicate significant differences compared with the results for the compound-free medium group ($P < 0.05$).

These results suggest that combination therapy with miconazole and 2-(4-methylpiperazin-1-yl)cyclopentanol may reduce miconazole dose. The limitation of this study is that no *in vivo* assays and toxicity tests have been performed to assess the efficacy of this compound. Therefore, it may be difficult to adapt this compound to animals and humans immediately. In the future, it is necessary to perform these tests. Furthermore, the effect of this compound on other fungal species should be investigated and the mechanism of action should be investigated in detail.

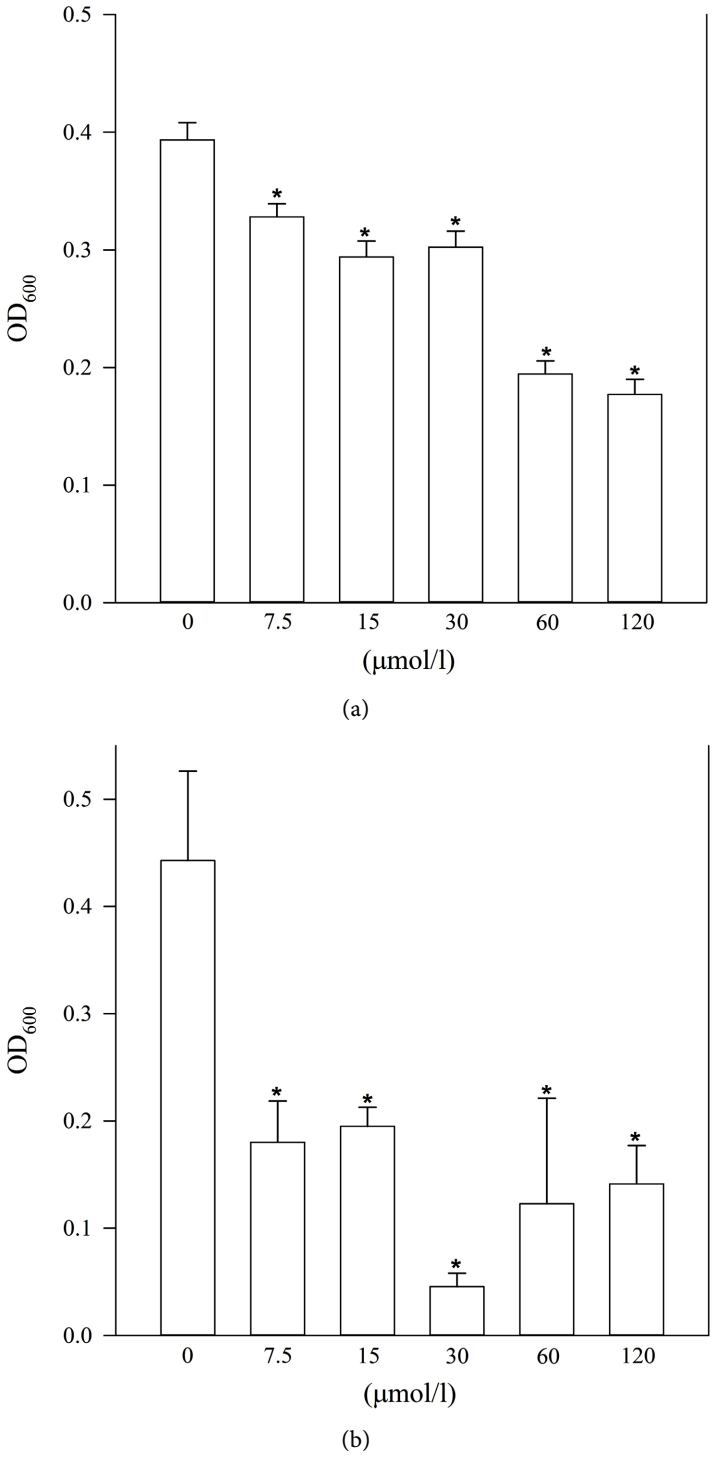

Figure 3. The growth inhibitory effect of *Candida albicans* ATCC 10231 by the combined use of 2-(4-methylpiperazin-1-yl)cyclopentanol (10 mmol/l) and varying amounts of miconazole. (a) Growth in the 2-(4-methylpiperazin-1-yl)cyclopentanol-free medium. (b) Growth in the 2-(4-methylpiperazin-1-yl)cyclopentanol-added medium. The data are presented as the mean ± SD (n = 3). Asterisks indicate significant differences compared with the results for the miconazole-free medium group ($P < 0.05$).

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Conflicts of Interest

The author declares no conflicts of interest regarding the publication of this paper.

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