Screening of Compounds from an FDA-Approved Drug Library for the Ability to Inhibit Aspartic Protease Secretion from the Pathogenic Yeast *Candida albicans*


Department of Microbiology, Meiji Pharmaceutical University, 2-522-1 Noshio, Kiyose, 204-8588 Japan

**Abstract**

The pathogenic fungus *Candida albicans* causes disseminated candidiasis with a poor prognosis in immunocompromised hosts. Secreted aspartyl protease (Sap) from the microorganism acts as a hydrolase to facilitate invasion into host tissues. Inhibition of *Candida* Sap activity could be a new treatment strategy for candidiasis. In the present study, we screened compounds from an FDA-approved drug library, Screen-Well™, for their ability to inhibit *Candida* Sap activity. Sixteen compounds (piroxicam, carbidopa, nisoldipine, cerivastatin, fluvastatin, mycophenolic acid, rapamycin, bleomycin, bortezomib, 5-fluorouracil, floxuridine, fumagillin, pentamidine, albendazole, fenbendazole, and amphotericin) inhibited Sap activity in a dose-dependent manner in vitro, although strain differences in the activity of the compounds were observed. Our study shows that existing drug compounds have the potential to inhibit Sap activity.

**Keywords**: *Candida albicans*; Secreted aspartyl protease; Inhibitor; FDA-approved drug library

**Introduction**

Although the yeast-like fungus *Candida albicans* colonizes mucosal surfaces in healthy individuals, it is capable of causing disseminated candidiasis from mucosal infection in immunocompromised hosts. Disseminated candidiasis is life-threatening and its prognosis is very poor [1-3]. To cause an infection, the pathogen invades host tissues. *C. albicans* secretes aspartic protease (Sap) as a hydrolase into the host tissue [4-5]. *C. albicans* Sap plays an important role in not only host tissue invasion but also inactivation of complement, defensin, and lactoferrin, which are involved in host defense.

*C. albicans* has 10 Saps (Sap1 to Sap10). Sap1, Sap2, Sap3, Sap8, Sap9, and Sap10 are produced mainly by the yeast form, while Sap4, Sap5, and Sap6 are produced mainly by the hyphal form [6,7]. Their optimal pH range is 3 to 5. In an animal model, the survival rate of animals infected with *SAP* gene-disrupted cells was significantly higher than that of those infected with wild-type cells [8]. Therefore, it is obvious that Sap is a major virulence factor in *C. albicans*.

Antifungal drugs—including amphoterin B, azole derivatives, and echinocandin—have been widely used to treat candidiasis; however, the number of antifungal drugs is small compared to that of antibacterial or antiviral drugs. As fungi are eukaryotic organisms, like humans, achievement of selective toxicity against fungal cells is more difficult compared to bacteria or viruses. In addition, the number of drug-resistant *C. albicans* clinical isolates has increased [9-11].

During the last decade, various drugs targeted to virulence factors of fungi have been developed. The structure of *C. albicans* Sap2 and HIV protease are similar, which likely explains the fact that adhesion of *C. albicans* to tissues was reduced by HIV protease inhibitors (PIs) [12].

In the present study, we identified compounds in an FDA-approved drug library that function as Sap inhibitors.

**Materials and Methods**

**Strains examined**

Four *C. albicans* strains (J2-36, J2-40, J2-73, and J2-80) were examined in this study. They were isolated from the blood of candidiasis patients and are resistant to fluconazole, itraconazole, and voriconazole. Strains were maintained on Sabouraud dextrose agar (2% glucose, 1% polyplpeptone, 0.5% yeast extract, 1.5% agar) at 37°C.

**Screening of sap inhibitors**

Extracellular proteinase activity was measured using a spectrophotometric method [13]. Yeasts and drugs were incubated in YYY medium (1.17% yeast carbon base, 0.01% yeast extract, 0.27% glucose) at 37°C for 48 h. Then, 0.1 mL of supernatant was added to 0.9 mL of 0.1 M citrate buffer (pH 3.2) containing 0.2% bovine serum albumin (BSA) and incubated at 37°C for 48 h. The reaction was terminated after 3 min by adding 5% trichloroacetic acid. The mixture was centrifuged, and the absorbance at 280 nm of the supernatant was measured. Cell-free and drug-free controls were included. The experiment was conducted in triplicate. The supernatants were also analyzed by SDS-PAGE.

**Drug susceptibility testing**

If a compound inhibited the growth of *C. albicans*, its Sap inhibitory activity could not be evaluated. Therefore, the MIC of each

*Corresponding author: Sugita T, Department of Microbiology, Meiji Pharmaceutical University, Nooshi, Kiyose, Tokyo 204-8588, Japan, Tel: +81-424-95-8762; E-mail: sugita@my-pharm.ac.jp

Received August 21, 2014; Accepted September 16, 2014; Published September 19, 2014


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drug against \textit{C. albicans} strains was determined according to the CLSI 27A3 guidelines [14].

**Results**

**Inhibitory effect**

Fifteen antifungal drugs included in the library of 640 FDA-approved drugs were excluded. The tested concentration was chosen on the basis of information from Kaneko et al. [15] and our MIC testing. Sixteen compounds that showed an inhibitory effect of >0.2 are shown in Table 1. They showed dose-dependent Sap inhibitory activities (Figure 1). The tested concentration of each compound is also shown in Table 1. We confirmed that the tested concentration of each compound did not inhibit the growth of \textit{C. albicans}. Strain differences were observed in the inhibition of \textit{Candida} Sap activity. For example, the immunosuppressive agent rapamycin had an inhibitory effect of 0.9 on the Sap activity of two strains, and an inhibitory effect of 0.1 on that of two other strains (Figure 1). The Sap inhibitory effects of the compounds were confirmed by SDS-PAGE. A representative image of an SDS-PAGE gel is shown in Figure 2. Fenbendazole inhibited BSA degradation in a dose-dependent manner. No BSA fragments were detected in the drug-free supernatant.

**Discussion**

The number of antifungal drugs is limited and the number of antifungal drug-resistant fungi is increasing. Also, the number of immunocompromised patients has increased despite improvements in medical technology. Thus, development of new drugs based on novel mechanisms is required. During the last decade, treatments targeting virulence factors of pathogenic fungi have been investigated. The most intensively investigated such drug target is Sap. Since HIV PIs can also inhibit or reduce \textit{Candida} Sap activity, Sap inhibitors may represent new antifungal drugs [16-20]. Ritonavir, saquinavir, and amprenavir inhibited Sap activity, whereas indinavir and nelfinavir did not. Amprenavir had particularly strong activity (85 to 100% at 6.25 to 200 mol/L) [21]. The FDA-approved drug library includes three HIV PIs: amprenavir, atazanavir, and nelfinavir. The latter two compounds showed no inhibitory effect, however. Our study also confirmed that amprenavir had inhibitory activity at 10 μg/mL (17.6 μM). HIV PI screening has been performed using a library of small-molecule peptidomimetics [22].

**Table 1**: Compounds that showed Sap inhibitory activity against \textit{C. albicans} strains.

<table>
<thead>
<tr>
<th>MIC (μg/mL)</th>
<th>Concentration used in this study</th>
<th>Therapeutic category</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;10</td>
<td>10, 1, 0.1</td>
<td>Anti-inflammatory drug, NSAID</td>
</tr>
<tr>
<td>&gt;10</td>
<td>10, 1, 0.1</td>
<td>Anti-Parkinson drug</td>
</tr>
<tr>
<td>&gt;10</td>
<td>10, 1, 0.1</td>
<td>Anti-hypertensive drug</td>
</tr>
<tr>
<td>5</td>
<td>5, 0.5, 0.05</td>
<td>Anti-hyperlipidemia drug</td>
</tr>
<tr>
<td>&gt;10</td>
<td>10, 1, 0.1</td>
<td>Anti-hyperlipidemia drug</td>
</tr>
<tr>
<td>&gt;0.15</td>
<td>0.1, 0.01, 0.001</td>
<td>Immunosuppressive drug</td>
</tr>
<tr>
<td>&gt;1.25</td>
<td>0.1, 0.01, 0.001</td>
<td>Immunosuppressive drug</td>
</tr>
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<td>&gt;10</td>
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<td>Anti-tumor drug</td>
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<td>Anti-tumor drug</td>
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<tr>
<td>5-10</td>
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<td>Anti-protozoal drug</td>
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<tr>
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<td>10, 1, 0.1</td>
<td>Anti-echinococcosis drug</td>
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<tr>
<td>&gt;10</td>
<td>10, 1, 0.1</td>
<td>Anti-nebotadole drug</td>
</tr>
<tr>
<td>&gt;10</td>
<td>10, 1, 0.1</td>
<td>Anti-virus drug</td>
</tr>
</tbody>
</table>

**Figure 1**: Sap inhibitory effect against four \textit{C. albicans} strains.

The inhibitory effect was evaluated at 48 h and calculated as follows: Inhibitory effect = (\text{Abs of B} - \text{Abs of C}) / (\text{Abs of D} - \text{Abs of C}), where \text{Abs of A} is the absorbance at 280 nm, \text{B} is cells plus compound, \text{C} is compound only (no cells), and \text{D} is cells only (no compound). Red circle, strain J2-36; blue square, strain J2-40; green diamond, strain J2-73; green triangle, strain J2-80.
The present study revealed that in addition to one HIV PI, 15 other chemically and pharmacologically diverse compounds had inhibitory effects on Candida Sap activity. As there is no structural similarity between these 15 compounds and HIV PIs, the inhibitory mechanism of these 15 compounds may differ from that of HIV PIs. Our preliminary study indicated that in the presence of fenbendazole Sap2p was not detected in supernatants by western blotting analysis using an anti-Sap2p monoclonal antibody, suggesting that fenbendazole inhibits production of Sap2p (unpublished data).

The increasing number of antifungal drug-resistant C. albicans strains is clinically problematic. Virulence inhibitors—such as those active against Sap—are required to be effective against antifungal drug-resistant strains; therefore, we used azole-resistant strains in this study.

In conclusion, we identified compounds other than HIV PIs that inhibited Candida Sap activity in an FDA-approved drug library. Although these compounds are not immediately useful for treatment of candidiasis because of inadequate knowledge of the appropriate dosage and administration routes and possible interactions with other medicines, they may be used as lead compounds to develop new antifungal agents targeting virulence factors.

Acknowledgements

This study was supported in part by a grant from the Ministry of Health, Labour and Welfare of Japan (H26-shinkoujitsuyoka-ippan-010).

References