

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

Cho O, Shiokama T, Ando Y, Aoki N, Uehara C, Maeda E, Matsumoto S, Kurakado S and Sugita T*

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 amprenavir) 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 Sap 4, 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 amphotericin 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% polypeptone, 0.5% yeast extract, 1.5% agar) at 37°C.

Reagents

An FDA-approved drug library, Screen-Well™, Japanese version (Cosmo-Bio, Tokyo, Japan), was used for the screening study. The drugs were dissolved in dimethyl sulfoxide (DMSO) and adjusted to a concentration of 1 mg/mL. The library consists of 635 drugs, which are listed in Table 1.

Screening of sap inhibitors

Extracellular proteinase activity was measured using a spectrophotometric method [13]. Yeasts and drugs were incubated in YYG 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, Noshio, 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

Citation: Cho O, Shiokama T, Ando Y, Aoki N, Uehara C, et al. (2014) Screening of Compounds from an FDA-Approved Drug Library for the Ability to Inhibit Aspartic Protease Secretion from the Pathogenic Yeast *Candida albicans*. Pharmaceut Reg Affairs 3: 126. doi:10.4172/2167-7689.1000126

Copyright: © 2014 Cho O, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

MIC (ug/mL) ^{a)}	Concentration used in this study	Therapeutic category
>10	10, 1, 0.1	Anfi-inflammatory drug, NSAID
>10	10, 1, 0.1	Anti-Parkinson drug
>10	10, 1, 0.1	Anti-hypertensive drug
5	5, 0.5, 0.05	Anti-hyperlipidemia drug
>10	10, 1, 0.1	Anti-hyperlipidemia drug
>10	10, 1, 0.1	Immunosuppressive drug
>0.15	0.1, 0.01, 0.001	Immunosuppressive drug
>1.25	0.1, 0.01, 0.001	Anti-tumor drug
>10	10, 1, 0.1	Anti-tumor drug
>10	10, 1, 0.1	Anti-tumor drug
>10	10, 1, 0.1	Anti-tumor drug
>10	10, 1, 0.1	Anti-amebic dysentery drug
5-10	1, 0.1, 0.01	Anti-protozoal drug
>10	10, 1, 0.1	Anti-echinococcosis drug
>10	10, 1, 0.1	Anti-nebatode drug
>10	10, 1, 0.1	Anti-virus drug

Table 1: Compounds that showed Sap inhibitory activity against *C. albicans* strains.

drug against *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 *C. albicans*. Strain differences were observed in the inhibition of *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 *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].

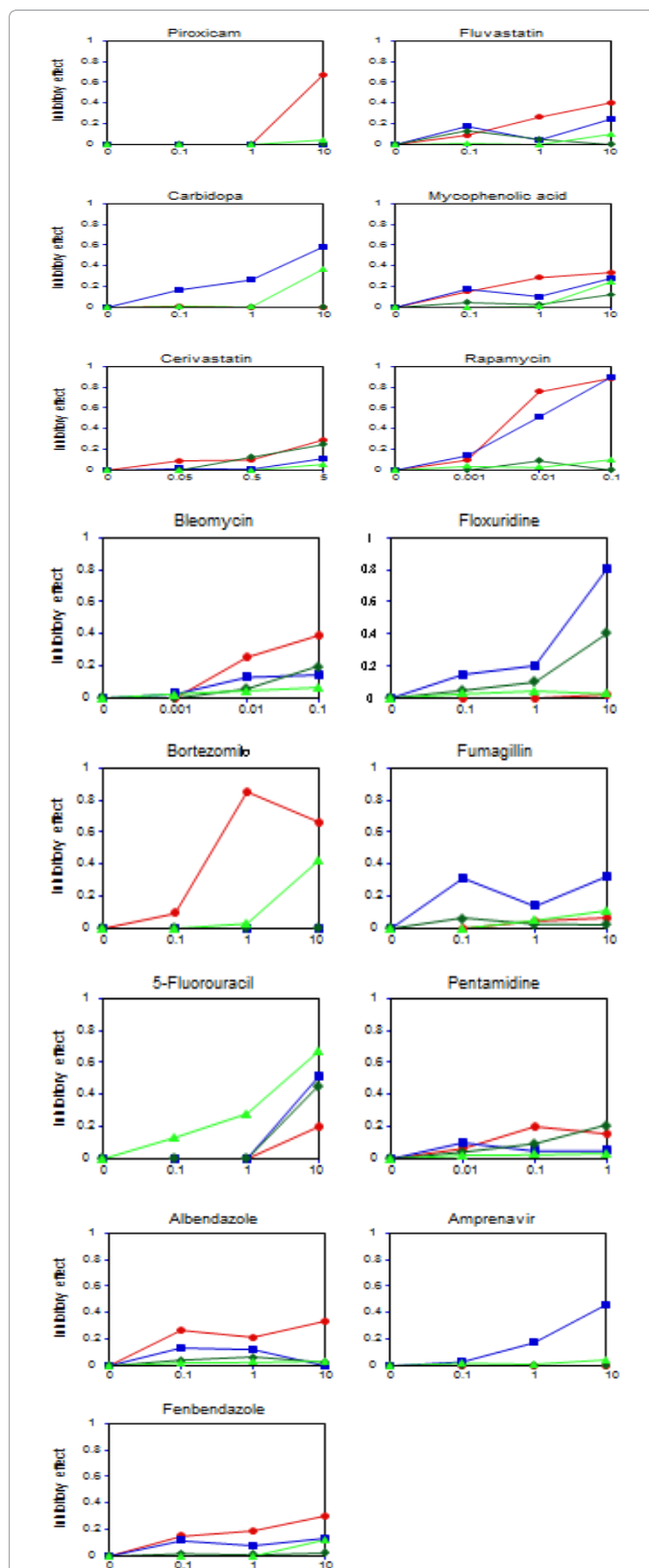


Figure 1: Sap inhibitory effect against four *C. albicans* strains. The inhibitory effect was evaluated at 48 h and calculated as follows: Inhibitory effect = $(A_{280} B - A_{280} C) / (A_{280} D - A_{280} C)$, where A_{280} is the absorbance at 280 nm, B is cells plus compound, C is compound only (no cells), and D is cells only (no compound). Red circle, strain J2-36; blue square, strain J2-40; green triangle, strain J2-73; green diamond, strain J2-80.

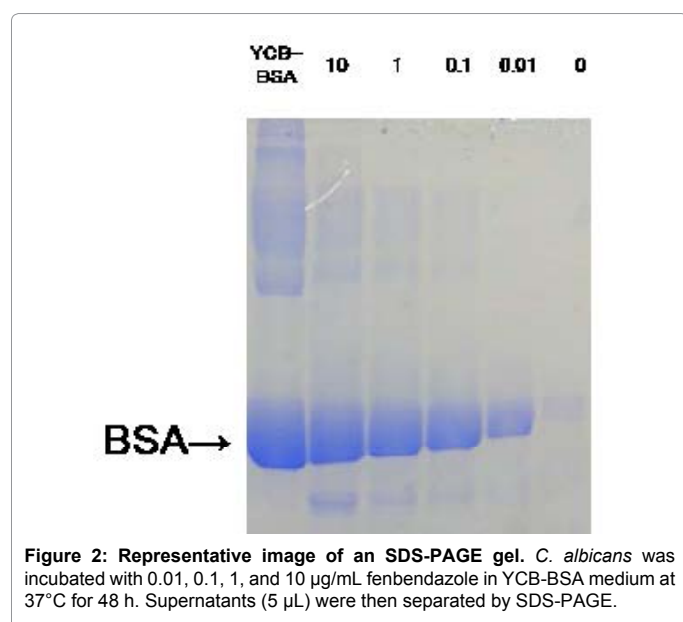


Figure 2: Representative image of an SDS-PAGE gel. *C. albicans* was incubated with 0.01, 0.1, 1, and 10 µg/mL fentendazole in YCB-BSA medium at 37°C for 48 h. Supernatants (5 µL) were then separated by SDS-PAGE.

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 fentendazole Sap2p was not detected in supernatants by western blotting analysis using an anti-Sap2p monoclonal antibody, suggesting that fentendazole 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

- Mavor AL, Thewes S, Hube B (2005) Systemic fungal infections caused by *Candida* species: epidemiology, infection process and virulence attributes. *Curr Drug Targets* 6: 863-874.
- Mayer FL, Wilson D, Hube B (2013) *Candida albicans* pathogenicity mechanisms. *Virulence* 4: 119-128.
- Naglik JR, Moyes DL, Wächtler B, Hube B (2011) *Candida albicans* interactions with epithelial cells and mucosal immunity. *Microbes Infect* 13: 963-976.
- Silva NC, Nery JM, Dias AL (2014) Aspartic proteinases of *Candida* spp.: role in pathogenicity and antifungal resistance. *Mycoses* 57: 1-11.
- Braga-Silva LA, Santos AL (2011) Aspartic protease inhibitors as potential anti-*Candida albicans* drugs: impacts on fungal biology, virulence and pathogenesis. *Curr Med Chem* 18: 2401-2419.
- Hube B, Naglik J (2001) *Candida albicans* proteinases: resolving the mystery of a gene family. *Microbiology* 147: 1997-2005.
- Braga-Silva LA, dos Santos AL, Portela MB, Souto-Padrón T, de Araújo Soares RM (2007) Effect of suramin on the human pathogen *Candida albicans*: implications on the fungal development and virulence. *FEMS Immunol Med Microbiol* 51: 399-406.
- Sanglard D, Hube B, Monod M, Odds FC, Gow NA (1997) A triple deletion of the secreted aspartyl proteinase genes SAP4, SAP5, and SAP6 of *Candida albicans* causes attenuated virulence. *Infect Immun* 65: 3539-3546.
- Pfaller MA, Messer SA, Woosley LN, Jones RN, Castanheira M (2013) Echinocandin and triazole antifungal susceptibility profiles for clinical opportunistic yeast and mold isolates collected from 2010 to 2011: application of new CLSI clinical breakpoints and epidemiological cutoff values for characterization of geographic and temporal trends of antifungal resistance. *J Clin Microbiol* 51: 2571-2581.
- Ben-Ami R, Rahav G, Elinav H, Kassis I, Shalit I, et al. (2013) Distribution of fluconazole-resistant *Candida* bloodstream isolates among hospitals and inpatient services in Israel. *Clin Microbiol Infect* 19: 752-756.
- Pfaller MA (2012) Antifungal drug resistance: mechanisms, epidemiology, and consequences for treatment. *Am J Med* 125: S3-13.
- Tsang CS, Hong I (2010) HIV protease inhibitors differentially inhibit adhesion of *Candida albicans* to acrylic surfaces. *Mycoses* 53: 488-494.
- De Bernardis F, Mondello F, San Millán R, Pontón J, Cassone A (1999) Biotyping and virulence properties of skin isolates of *Candida parapsilosis*. *J Clin Microbiol* 37: 3481-3486.
- Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; Approved Standard-Third Edition, M27-A3, Clinical and Laboratory Standards Institute.
- Kaneko Y, Fukazawa H, Ohno H, Miyazaki Y (2013) Combinatory effect of fluconazole and FDA-approved drugs against *Candida albicans*. *J Infect Chemother* 19: 1141-1145.
- Korting HC, Schaller M, Eder G, Hamm G, Böhmer U, et al. (1999) Effects of the human immunodeficiency virus (HIV) proteinase inhibitors saquinavir and indinavir on in vitro activities of secreted aspartyl proteinases of *Candida albicans* isolates from HIV-infected patients. *Antimicrob Agents Chemother* 43: 2038-2042.
- Cassone A, De Bernardis F, Torosantucci A, Tacconelli E, Tumbarello M, et al. (1999) In vitro and in vivo anticandidal activity of human immunodeficiency virus protease inhibitors. *J Infect Dis* 180: 448-453.
- Asencio MA, Garduño E, Pérez-Giraldo C, Blanco MT, Hurtado C, et al. (2005). Exposure to therapeutic concentrations of ritonavir, but not saquinavir, reduces secreted aspartyl proteinase of *Candida parapsilosis*. *Chemotherapy* 51: 252-255.
- De Bernardis F, Tacconelli E, Mondello F, Cataldo A, Arancia S, et al. (2004) Anti-retroviral therapy with protease inhibitors decreases virulence enzyme expression in vivo by *Candida albicans* without selection of avirulent fungus strains or decreasing their anti-mycotic susceptibility. *FEMS Immunol Med Microbiol* 41: 27-34.
- Cenci E, Francisci D, Belfiori B, Pierucci S, Baldelli F, et al. (2008) Tipranavir exhibits different effects on opportunistic pathogenic fungi. *J Infect* 56: 58-64.
- Braga-Silva LA, Mogami SS, Valle RS, Silva-Neto ID, Santos AL (2010) Multiple effects of amprenavir against *Candida albicans*. *FEMS Yeast Res* 10: 221-224.
- Tossi A, Benedetti F, Norbedo S, Skrbec D, Berti F, et al. (2003) Small hydroxyethylene-based peptidomimetics inhibiting both HIV-1 and *C. albicans* aspartic proteases. *Bioorg Med Chem* 11: 4719-4727.