SUPPLEMENTARY MATERIAL

***In vitro* avarol does affect the growth of *Candida* sp.**

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Abstract

This work extends *in vitro* screening of antimicrobial activity of avarol, the marine natural product firstly isolated from the Mediterranean sponge *Dysidea avara*. Its anticandidial activity was evaluated by microdilution method against eight *Candida* strains, two ATCC and six clinical ones. At a different extent this compound was proven to be active against all the strains tested (MIC 0.8-6.0 µg/mL and MFC 1.6-12.0 µg/mL, respectively). According to the best of our knowledge, this is the first report on avarol activity towards any yeast strain which may be of relevance for Alzheimer's disease. Indeed, avarol derivatives showing moderate acetylcholinesterase activity should be screened for anticandidial activity both *in vitro* and *in vivo*.

Keywords: Marine sponge, *Dysidea avara*, avarol, oral pathogens, anticandidial activity, Alzheimer's disease

Experimental

Biological material

Avarol was isolated from the sponge *Dysidea avara* Schmidt (Dictyoceratida), collected in the Bay of Naples, Italy (Minale et al. 1974; De Rosa et al. 1976).

Microorganisms

In antifungal assay, six clinical isolates of *Candida* spp. and two ATCC strains (*Candida albicans* ATCC 10231 and *Candida tropicalis* ATCC 750) were used. The reference

strains were obtained from the Laboratory of Mycology, Institute for Biological Research 'Sinisa Stankovic', University of Belgrade, Serbia. Strains of *Candida* spp. were maintained on Sabourand Dextrose Agar (SDA, Merck, Germany). All clinical oral isolates were obtained by rubbing a sterile cotton swab over oral mucosa from patients at the Department of Pediatric and Preventive Dentistry, Faculty of Dental Medicine, University of Belgrade, Serbia. The fungal colonies were properly identified (Nikolić et al. 2012).

Anticandidal activity

Minimum inhibitory (MIC) and minimum bactericidal/fungicidal (MBC/MFC) concentrations were determined by microdilution method in 96 well microtitre plates, described by Douk et al. (1995) and EUCAST (2002) with some modifications. Briefly, fresh overnight cultures of yeasts were adjusted with sterile saline to a concentration of 1.0×10^4 CFU per well. Avarol was then added in SDB medium (Merck, Germany). The microplates were incubated for 48 h at 37 °C. The MIC was defined as the lowest concentration of avarol inhibiting the visible growth of the test strain. The MIC values for the yeasts tested were detected following the addition of 40 µL of *p*-iodonitrotetrazolium violet (INT) 0.2 mg/mL (Sigma-Aldrich I8377, Munich, Germany) and incubation at 37 °C for 30 min (Tsukatani et al. 2012). The MFCs were determined by serial subcultivations of 10 µL into microtiter plates containing 100 µL of broth per well and further incubation for 24 h at 37 °C. The lowest concentration with no visible growth was defined as the MFC, indicating 99.5% killing of the original inoculum. The mycotic fluconazole (Hemofarm A.D., Vrsac, Serbia) was used as a positive control.

References

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