Antifungal Activity of Some Bis-5-methylbenzimidazole Compounds

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ABSTRACT. Twenty bis-5-methylbenzimidazole compounds were evaluated for their in vitro antifungal activity against Candida albicans and Candida tropicalis. Except for three all compounds exhibited an antifungal activity against these yeasts over a range of the minimum inhibitory concentration (MIC) between 25 and 800 μg/mL.

The incidence of fungal infections has increased significantly in the last few years (Del Poeta et al. 1998). For this reason, the development of new antifungal agents with potent and broad-spectrum fungicidal activity is very important. A large number of benzimidazole derivatives were shown to exhibit important biological properties, such as antifungal, antibacterial, antihelminthic, antiallergic, antineoplastics, local analgesic, antihistaminic, hypotensive, vasodilator, spasmolytic and antiulcer activities (Kuçükbay et al. 1995–1997, 2001; Güneş and Coar 1992; Carlsson et al. 2002; Gulyás et al. 2002; Ülküseven et al. 2002). In recent years, considerable attention has been given to the synthesis of bis-benzimidazole compounds because of their properties in cancer therapy. Various studies with bis-benzimidazole derivatives have shown that binding affinity to calf thymus DNA correlates positively with in vitro topoisomerase inhibitory potency or cytotoxicity (Turner et al. 1996). Bis-benzimidazole compounds also showed versatile pharmacological activities such as antifungal, antihelminthic, antiviral, anticoagulant and antiinflammatory activities (Hall et al. 1998). We have recently reported the synthesis of some bis-benzimidazole compounds and their selective in vitro antibacterial activity against standard bacterial strains, viz. Enterococcus faecalis ATCC 29212, Staphylococcus aureus ATCC 29213, Escherichia coli ATCC 25922 and Pseudomonas aeruginosa ATCC 27853 (Kuçükbay et al. 2004). Following our synthesis and antibacterial activity examination of the bis-benzimidazole compounds, we now report on the antifungal activity of those compounds against Candida albicans and C. tropicalis. The screening was carried out according to a macrobroth dilution reference method for in vitro antifungal susceptibility testing of yeast according to the National Committee for Clinical Laboratory Standards (NCCLS 1992).

MATERIALS AND METHODS

Chemistry. The reactions involve nucleophilic substitution of 5-methylbenzimidazole by appropriate butylene or but-2-enylene dihalides to obtain 1,4-bis(5-methylimidazol-1-yl)butane (1a) and 1,4-bis(5-methylimidazol-1-yl)but-2-ene (1b) followed by quaternization (halides 2a–11a and 2b–11b) (Scheme 1). 5-Methylbenzimidazole was synthesized from the reaction of 4-methylbenzene-1,2-diamine and formic acid similar to the benzimidazole synthesis (Vogel 1978). The purity of compounds was checked by 1H-, 13C-NMR (Bruker WM360; Bruker instruments, USA) and FT-IR (ATI Unicam, Mattson 1000; ATI/Mattson Instruments, UK), using also a melting point apparatus (Electrothermal 9200; Electrothermal Engineering, UK).

Biological activity. Antifungal activity of the compounds was determined by using the macrobroth dilution procedure (NCCLS 1992). Minimum inhibitory concentration (MIC) for each compound was determined against Candida albicans and C. tropicalis obtained from the Department of Microbiology, Faculty of Medicine, Ege University (Turkey). The stock solutions of compounds were prepared in dimethyl sulfoxide (Me2SO) which had no effect on the microorganism at the concentrations used. The final concentrations of tested compounds were 800, 400, 200, 100, 50, 25 and 12.5 μg/mL. Fluconazole in serial 2-fold
dilutions was used as a reference compound. Briefly, to estimate MIC, *C. albicans* and *C. tropicalis* after an overnight growth were suspended in 0.85 % NaCl; the cell concentration was adjusted to $10^5$ CFU/mL. One-hundred µL of this suspension was inoculated into tubes with compounds diluted in 0.9 mL of broth medium. After a 2-d incubation at 35 °C, growth was estimated. The MIC was determined as the lowest concentration of the compounds which provided a slightly hazy growth compared with that of the drug-free growth control.

![Scheme 1. Synthesis of the tested bis-5-methylbenzimidazole derivatives.](image)

**Scheme 1.** Synthesis of the tested bis-5-methylbenzimidazole derivatives.

### RESULTS AND DISCUSSION

The antifungal activity results (MIC values) are given in Table I. The compounds 1a, 2a, 4a, 5a, 7a–11a, 3b, 5b, and 7b–11b showed some activity in the MIC range of 25–400 mg/L against both *Candida* strains.

Compounds 7a–9a and 11b were the most effective (MIC 25–50 mg/L) against both strains. On the basis of these data it is suggested that the substituted aryl moiety may play an important role in the antifungal activity when attached via the 1- and 1’-position of the 5-methylbenzimidazole. As a result, the antifungal activity values of the tested compounds are much higher than fluconazole (reference compound); therefore, our compounds cannot be suggested for clinical use at this stage.