

Antimicrobial Effectiveness of Maleimides on Fungal Strains Isolated from Onychomycosis

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ABSTRACT

This study aimed to analyze the effectiveness of maleimides as inhibitors on the growth of fungal strains isolated from onychomycosis by the solid medium diffusion procedure. The results showed a promising antifungal activity of the assayed maleimides with formation of fungal growth inhibition halos oscillating between diameter 10 and 23mm. MIC was 100µg/mL for 3,4-dichloro-N-phenyl-methyl-maleimide and 3,4-dichloro-N-phenyl-propyl-maleimide and 200µg/mL for 3,4-dichloro-N-phenyl-maleimide, 3,4-dichloro-N-phenyl-ethyl-maleimide and 3,4-dichloro-N-phenyl-buthyl-maleimide.

Key words: Onychomycosis, fungi, maleimides, antifungal activity

INTRODUCTION

Onychomycosis is a kind of fungal infections in which nails are affected (Sampaio and Rivitti, 1999), more frequently feet nails representing 80% of the cases (Achten and Wanet-Rouard, 1998). Etiologically, approximately 90% of the onychomycosis is caused by dermatophyte fungi such as *Tricophyton rubrum*, *T. mentagrophytes* var. *interdigitale* and *Epidermophyton floccosum* (Graybill, 1992). Yeasts, mainly *Candida* genera and moulds (e.g. *Scytadilium*, *Acremonium* and *Cephalosporium*) also have been reported as potential onychomycosis agents causing (Juan and Virendra, 2000).

It has been reported that the use of commonly available pharmaceutical preparations applied in

onychomycosis treatment have been some times inefficient (Midgley and Moore, 1996), needing to a search for antifungal compounds which would be more effective, cheaper, with wide antifungal spectrum, short time of use and minimum side effects (Juan and Virendra, 2000).

Maleimides are obtained easily and have presented satisfactory profile to the development of new and efficient drugs (Aquino et al., 2003). These have been characterized as similar to the filantimide alkaloid obtained from *Phylantus sellowianus* of Euphorbiaceae family (Andricopulo et al., 1998). Some compounds inserted in maleimides group showed expressive pharmacological properties as analgesic, anti-spasmodic, antibacterial and antifungal (Cechinel Filho et al., 1994; Santos et al., 1994; Cruz et al., 1996; Corrêa et al., 1997).

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The aim of this study was to evaluate the effectiveness of some maleimides on the growth of fungal strains isolated from onychomycosis.

MATERIALS AND METHODS

Maleimides

Antifungal action of 3,4-dichloro-*N*-phenyl-maleimide, 3,4-dichloro-*N*-phenyl-methyl-maleimide, 3,4-dichloro-*N*-phenyl-ethyl-maleimide, 3,4-dichloro-*N*-phenyl-propyl-maleimide and 3,4-dichloro-*N*-phenyl-buthyl-maleimide was analyzed at concentrations of 200, 100, 50, 25, 12.5, 6.3 and 3.1 µg/mL. The solutions were prepared in sterile distilled water prior to assays. Molecular structures of these are shown in Fig. 1.

Fungal strains

Candida albicans, *C. tropicalis*, *C. krusei*, *Trichophyton rubrum*, *T. mentagrophytes* and *Geotrichum candidum* strains were used as test microorganisms and obtained from clinical samples collected from patients infected with onychomycosis. Their isolation and identification were carried out according to standard procedures (Rebel and Taplin, 1974; Van-Rij, 1984; Hoog and Guarro, 1995).

Antifungal assay

Solid medium diffusion procedure using wells in dishes was used to evaluate the antifungal activity (Hadacek and Greger, 2000). Fungal inoculum of approximately 10^6 CFU/mL standardized by McFarland Scale 0.5 tube) and incubation time of 48 hours at 37°C for yeasts and 10-14 days/28°C for moulds were used (Pemplel et al., 1986). At the end of the incubation time, the fungal growth inhibition halos diameters were measured in millimeters using calipers. Minimum Inhibitory Concentration - MIC was considered the smallest maleimide concentration able to develop fungal growth inhibition halo equal or higher than 10mm diameter (Lima et al., 1993; Lima et al., 1999). Control assays were performed with ketoconazole (50 µg/mL). All assays were performed twice and the results were expressed as average.

RESULTS AND DISCUSSION

Infections caused by fungi including those of dermatological interest have shown increasing occurrence since 1980's, which has led to search for alternative substances with efficient antifungal properties and little or no toxicity to host (Graybill, 1992; Hazen, 1995). This high occurrence has been inducing factor for studies regarding the antifungal effectiveness of different chemical compounds on dermatological infections etiological agents (Aquino et al., 2003). The results regarding the sensitivity of fungal strains isolated from onychomycosis to maleimides are shown in Table 1. All assayed fungal strains were sensitive to maleimides, 3,4-dichloro-*N*-phenyl-methyl-maleimide and 3,4-dichloro-*N*-phenyl-propyl-maleimide which showed the smallest MICs. MIC was 200 µg/mL for 3,4-dichloro-*N*-phenyl-maleimide, 3,4-dichloro-*N*-phenyl-ethyl-maleimide and 3,4-dichloro-*N*-buthyl-maleimide; and 100 µg/mL for 3,4-dichloro-*N*-phenyl-methyl-maleimide and 3,4-dichloro-*N*-phenyl-propyl-maleimide. Fungal growth inhibition halos diameter were between 10mm (*G. candidum* x 3,4-dichloro-*N*-phenyl-methyl-maleimide, *G. candidum* x 3,4-dichloro-*N*-phenyl-ethyl-maleimide, *G. candidum* x 3,4-dichloro-*N*-buthyl-maleimide) and 23mm (*T. mentagrophytes* x 3,4-dichloro-*N*-phenyl-buthyl-maleimide).

Resistant behavior in *C. tropicalis* and *T. mentagrophytes* to the standard antifungal (Ketoconazole, 50 µg/mL) applied as positive control was observed. *T. mentagrophytes* was the most sensitivity strain with inhibition halos average diameter of 17.2mm. *G. candidum* was the least sensitive strain showing inhibition halos average diameter of 10.8mm.

Lima et al. (1999) found prominent antimicrobial activity in imidic compounds which were effective to inhibit the growth of *Escherichia coli*, *Staphylococcus aureus*, *Candida albicans*, *Microsporium canis* and *Penicillium*. Dantas et al. (2000) evaluated the sensitivity of *Candida* species and dermatophytes to maleimides, naftalimides and succimides and noted that only maleimides showed promising results as *Candida* inhibitor, while the inhibition of *Microsporium* and *Trichophyton* was similar for the three assayed compounds.

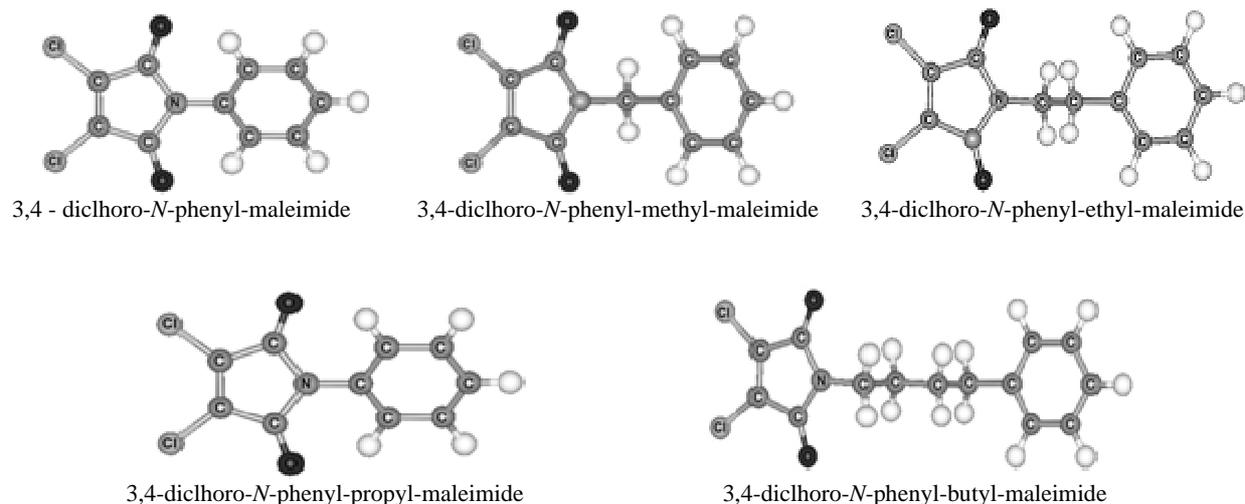


Figure 1 - Maleimides molecular structure.

Table 1 - Sensitivity of fungal strains isolated from onychomycosis to maleimides as measured in inhibition halos diameter (mm).

Maleimides	MIC ^a	Fungal strains				
		<i>C. albicans</i>	<i>C. tropicalis</i>	<i>T. rubrum</i>	<i>T. mentagrophytes</i>	<i>G. candidum</i>
3,4-dichloro- <i>N</i> -phenyl-maleimide	200	11	16	14	16	11
3,4-dichloro- <i>N</i> -phenyl-methyl-maleimide	100	12	12	16	15	10
3,4-dichloro- <i>N</i> -phenyl-ethyl-maleimide	200	14	14	12	16	10
3,4-dichloro- <i>N</i> -phenyl-propyl-maleimide	100	17	13	12	16	10
3,4-dichloro- <i>N</i> -phenyl-butyl-maleimide	200	17	13	12	23	13
Ketoconazole (50µg/mL)		22	R	10	R	10
Strain viability ^b		+	+	+	+	+

^a: expressed in µg/mL; ^b: fungal growth in Sabouraud agar without adding no maleimide or standard antifungal; R: resistant.

Cechinel Filho et al. (1994) and Cruz et al. (1996) carried out study regarding the antibacterial activity of cyclic amides, including *N*-phenyl-maleimides and *N*-aryl-dichloro-maleimides on *S. aureus*, *Salmonella tiphymurium* and *E. coli* and reported that only maleimides were able to inhibit these microorganisms presenting MIC between 200 and 50µg/mL. Studies regarding the molecular structure - biological activity relationship of maleimides have showed that their antimicrobial activity possibly could be related with the double bound in the imidic ring or with the nitrogen atom next to the benzene ring, which could lead to

electronic interactions between maleimides and microbial cells (Cechinel Filho and Yunes, 1998; Dantas et al., 2000). Moreover, increasing electronic density on nitrogen atom suggests participation in the activation of redox cycles that play major role for development of biological activities (Andricopulo et al., 1998).

The results obtained in our study showed prominent effectiveness of maleimides in inhibiting the growth of onychomycosis etiological agents. These findings and the emergent necessity of developing new and efficient antifungal compounds could lead to a thought regarding the

possible rational inclusion of these compounds in pharmaceutical compositions used for the anti-onychomycosis therapy.

RESUMO

Este estudo objetivou analisar a efetividade de maleimidas como inibidores do crescimento de cepas fúngicas isoladas de onicomicoses através da técnica de difusão em meio sólido. Os resultados mostraram destacável atividade antifúngica das maleimidas ensaiadas com a formação de halos de inibição do crescimento fúngico com diâmetros oscilando entre 10 e 23mm. A CIM encontrada foi 100µg/mL para 3,4-dicloro-*N*-fenil-metil-maleimida e 3,4-dicloro-*N*-fenil-propil-maleimida e 200µg/mL para 3,4-dicloro-*N*-fenil-maleimida, 3,4-dicloro-*N*-fenil-etil-maleimida e 3,4-dicloro-*N*-fenil-butil-maleimida.

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