

Study of Cytotoxic Activity of Neolignans 8.O.4', Arylpropanoids and Related Compounds with Antifungal Properties

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SUMMARY. The cytotoxic effects of neolignans 8.O.4', arylpropanoids and structurally related compounds are reported. Compounds having a strong antifungal activity, in parallel possess a potent cytotoxic effect, very similar to those displayed by amphotericin B and ketoconazole. Our results indicate that the actual role of arylpropanoids acting as antifungal compounds could be limited for topical use. However the strong antifungal activity and the novel mechanism of action of these arylpropanoids open the possibility of a promising utilization in agriculture and veterinary.

RESUMEN. "Estudio de la Actividad Citotóxica de Neolignanos 8.O.4', Arilpropanoides y Compuestos Relacionados con Propiedades Antifúngicas". Se reportan las actividades citotóxicas de neolignanos del tipo 8.O.4', arilpropanoides y compuestos estructuralmente relacionados. Aquellos compuestos que poseen una fuerte actividad antifúngica presentaron paralelamente una importante actividad citotóxica, ambas comparables a las que mostraron anfotericina B y ketoconazol. Nuestros resultados indican que el verdadero rol de los arilpropanoides actuando como compuestos antifúngicos podría estar limitado a un uso tópico. La fuerte actividad antifúngica y el novedoso mecanismo de acción de estos fenilpropanoides abren la posibilidad de una promotoria utilización en agricultura y veterinaria.

INTRODUCTION

The development of efficacious antifungal agents has not been as successful as that of antibacterial agents. The main reason is that fungi and mammals are both eukaryotes and their metabolic pathways are not significantly different. Consequently, it is difficult to develop new selective antifungal agents. However, the need for new antifungal agents is due to the increase of fungal infections in particular in immunocompromised hosts¹.

There is a vast literature dealing with the treatment of mycotic infections in normal and immunocompromised patients²⁻⁴. Thus, there appears to be a large and impressive array of drugs for the treatment of fungal infections. Unfortunately, the reality is quite different. There are, in fact, only very limited therapeutic options. Most of the available drugs have signifi-

cant and potentially life threatening side effects.

In the course of our screening program for antifungal activity, we report that 8.O.4' neolignans possess moderate but significant antifungal activity against dermatophytes^{5,6}. Considering that neolignans are plants compounds formed by two C₆-C₃ units, and dimerization of phenylpropanoids through dehydrogenation, produces the skeleton of all natural and synthetic lignans known to date, we carried out a systematic study of the antifungal properties of their phenylpropanoid moieties plus several structurally related compounds⁷. In that paper, we reported a strong antifungal activity of phenylpropanoids against dermatophytes. The antifungal effect of these compounds was comparable to those of amphotericin B and ketoconazole.

To gain insight into the mode of action of

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arylpropanoids, we performed an exhaustive conformational and electronic study on these compounds ⁸⁻¹⁰. Our results indicate that keto-enol tautomerization may be one of the mechanism of antifungal activity.

It is clear that the toxicity of any new potential antifungal agent is a key factor for the future perspectives of this class of compounds. Thus in the present work we report the cytotoxic effect of nine arylpropanoids and three neolignans 8.0.4', in order to determine the actual capacities and limitations of these compounds acting as antifungal agents.

MATERIALS AND METHODS

Cell culture

The lymphocytes were isolated from the spleen of Holfman rats (100-200 g), in sterile conditions ¹¹. The erythrolysis was performed in NH₄Cl solution.

The lymphocytes were cultured at 3 x 10⁵ cells in 1 ml of RPMI (Sigma) supplemented with NaHCO₃ and fetal calf serum (FCS, Gibco) from 5 to 10%, as well as penicillium (100 U/ml) and streptomycin (100 µg/ml).

The cultures were incubated for 24 h at 37 °C, in 5% CO₂ atmosphere. After washing, the cells were transferred to a fresh medium supplemented with FCS and phytohaemagglutinin (PHA) ¹¹⁻¹³ and then incubated at 37 °C for other 48 h.

To assess the antiproliferative effects of arylpropanoids, 1 ml of RPMI with 3 x 10⁵ cells/ml lymphocyte suspension, was added with 10 µg/ml of compound. Cells cultured in presence

of colchicine (10 µg/ml) were used as control. After 48 h, 2 µCi/ml of [³H] thymidine (sp activity 2 Ci/min) (NEN) were added to each well and cultured for 4 h.

Harvesting culture

The cells of each well were collected in a fibre-glass filter disc (Whatman FG/A) with saline, then washed with 5% trichloroacetic (TCA) and finally with MeOH. A needle was then used to transfer the filter disc to glass beta vial; 10 ml of liquid scintillation counter were added and the dpm of incorporated thymidine were counted in a beta scintillation counter (Beckman). Results were expressed as dpm count in the test culture/dpm count in the control culture.

RESULTS AND DISCUSSION

Figures 1 and 2 show the cytotoxic activity obtained for arylpropanoids [1-8]. Phenylpropanoids [1-3] inhibited almost completely the cell growth (Figure 1). A closely related result was obtained for the naphthyl and phenanthryl compounds [4-8] (Figure 2). In contrast, the cytotoxic activity obtained for antifungal neolignans 8.0.4' [9-11] (Figure 3) was very weak in comparison with those displayed by the arylpropanoids with the same type of properties.

It is interesting to note that those compounds having a strong antifungal activity, in parallel possess a potent cytotoxic effect. This is particularly evident after examining both antifungal and cytotoxic results summarised in Table 1.

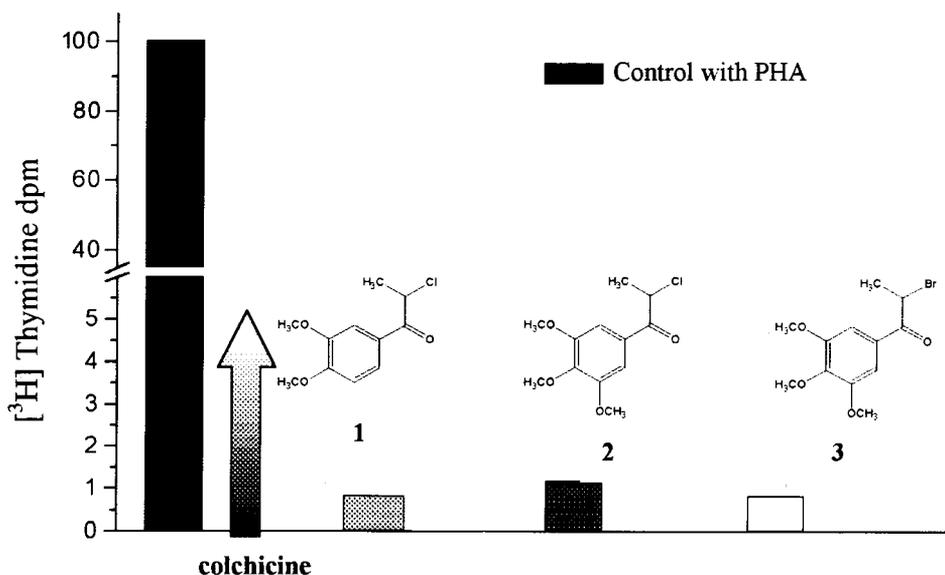


Figure 1. Cytotoxic activities obtained for phenylpropanoids [1-3]. Colchicine was included in the same figure as reference compound.

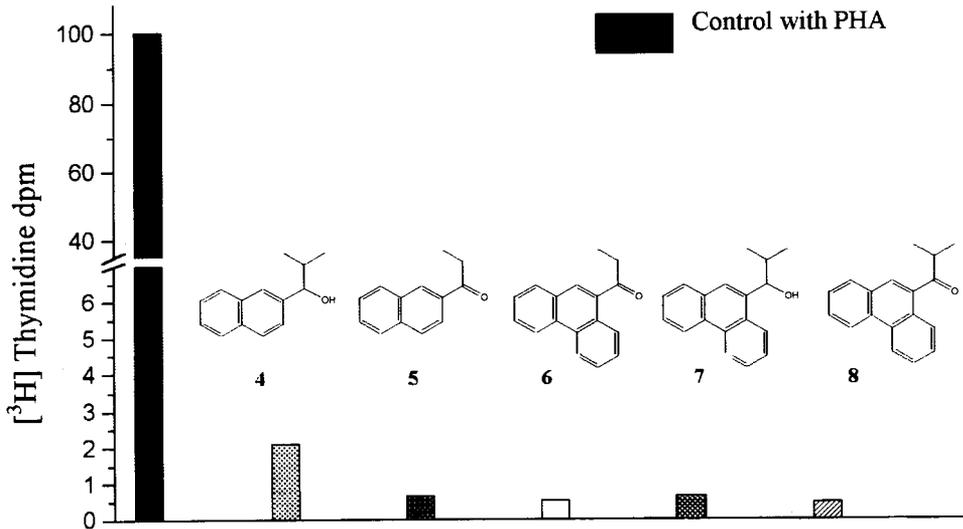


Figure 2. Cytotoxic activities obtained for arylpropanoids: naphthyl compounds [4-5] and phenanthryl derivatives [6-8].

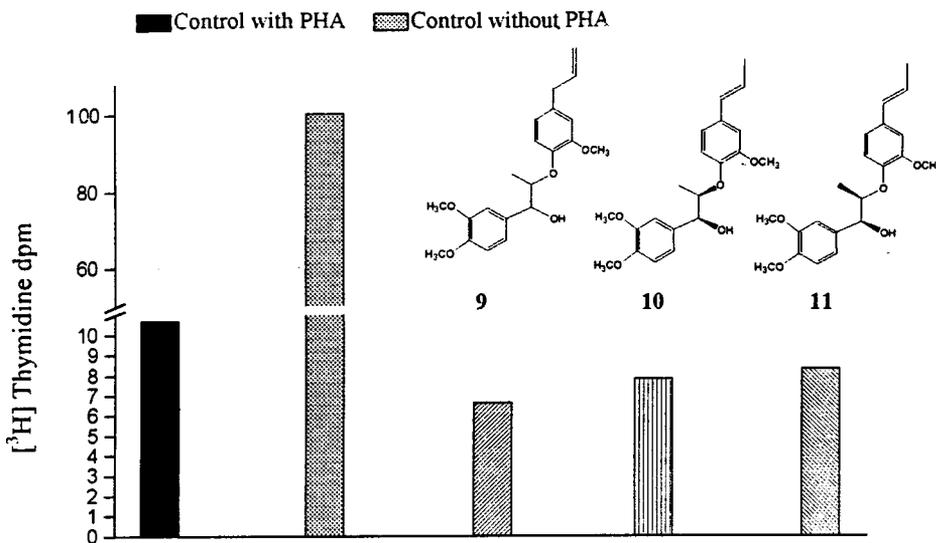


Figure 3. Cytotoxic activities obtained for antifungal neolignans 8.O.4' [9-11].

Compound	Cytotoxicity activity (dpm)	Antifungal activity MIC (µg/ml)		
		<i>T. rubrum</i>	<i>E. floccosum</i>	Reference
1	0.89	10	0.15	7
2	1.3	10	0.5	7
3	0.86	> 50	8	7
4	2	40	40	7
5	0.6	40	40	7
6	0.55	6.25	20	7
7	0.6	10	10	7
8	0.55	20	20	7
9	7	20	25	5
10	8	20	70	5
11	8.5	> 250	100	5
Ketoconazole	0.6	15	15	
Amphotericin B	1.8	25	0.3	
Colchicine	5	-		

Table 1. Cytotoxic activities obtained for arylpropanoids [1-8] and neolignans 8.O.4' [9-11]. *In vitro* antifungal activity taken from references 5 and 7 are also shown.

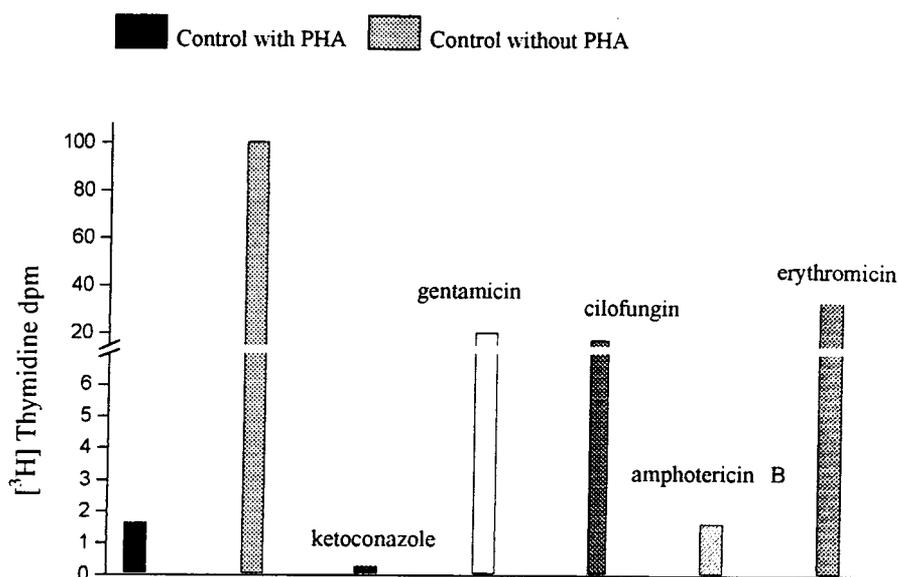


Figure 4. Cytotoxic activities obtained for antifungal and antibacterial agents currently in use: ketoconazole, gentamicine, cilofungin, amphotericin B and erythromycin.

In order to compare the cytotoxic activity of the compounds tested with those of antifungal and antibacterial agents currently in use, we evaluate the cytotoxic activity of amphotericin B and ketoconazole using the same bioassay (Figure 4). By comparing Figure 4 with Figures 1 and 2, it is clear that the cytotoxic effects of both ketoconazole and amphotericin B are comparable to those obtained for the arylpropanoid compounds and their derivatives.

Our results indicate that arylpropanoids reported here, possess a considerable cytotoxic activity. Even though their toxicities are similar to the antifungal agents amphotericin B and ketoconazole currently in use, the actual role of these arylpropanoids acting as antifungal agents, could be limited, since the specificity is critical in the treatment of mycoses. Nevertheless, the

fact that antifungal arylpropanoids [1-8] act by a novel mechanism of action, open the possibility of using them in agriculture or veterinary or eventually as model compounds for developing new derivatives with lower toxicities.

On the other hand, regarding antifungal neolignans 8.0.4', their low toxicities make them promisory compounds for developing new and safer antifungal agents on this structural basis.

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