Evaluation of anti-microbial potential of 3-ethenylcoumarins and their precursors

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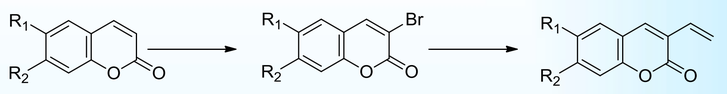
Introduction

Coumarins, classified as benzo-a-pyrones, are part of a large class of secondary phenolic metabolites derivatived from cinnamic acid, widely distributed in the plant kingdom but can also be found in fungi and bacteria. Today there are more than 1300 identified structures. In some plants they are mainly accumulated in the leaves, inhibiting the growth of pathogenic fungi and repelles insects. Coumarins are nowadays very important organic compounds due to their wide applicability resulting from their characteristic smell, therapeutic and biological activity.[1, 2].The changes in coumarin structure can provide new classes of compounds. The importance of the biological activity studies of these new compounds can be justified by what is observed in nature, a variety of biological activities in plants and animals, low toxicity and their presence in our diet (fruits and vegetables) as well as in some drugs. In this contest, eight coumarins (**Figure 1**) were tested. The anti-microbial properties of these coumarins were evaluated.[3,4] In a first approach four filamentous fungi (*Fusarium oxysporium* (CCMI866), *Aspergillus niger* (CCMI296), *Cladosporium* 7F1, *Trichoderma* sp (CCMI783)) was tested to access the antimicrobial spectrum of active compounds.



A - R_1=H, R_2=OH
B - R_1=OH, R_2=OH
C - R_1=H, R_2=OCH_3
D - R_1=OCH_3, R_2=OCH_3

E - R_1=H, R_2=OCH_3
F - R_1=OCH_3, R_2=OCH_3

G - R_1=H, R_2=OCH_3
H - R_1=OCH_3, R_2=OCH_3

Figure 1 – Coumarin derivatives.

Materials and Methodes

Table 1 – Tested compounds structure and the used concentrations.

	A	B	C	D	E	E	F	F	G	H
Concentration (mM)	5	5	5	5	50 5	50 5	5	5	5	5

1 Antifungal paper disks diffusion assay[3,4]

The fungi culture were incubated in a slat contaning Malt Extract Agar (MEA) at 25°C for 7 day.

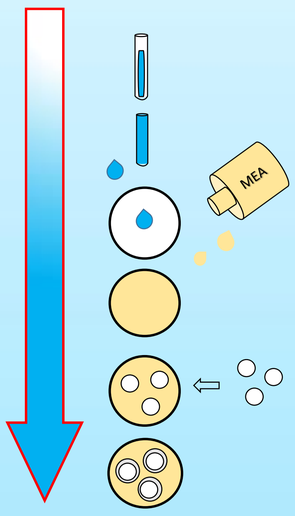
A diluted suspension was obtained.

The fungal suspensions was incorpored in MEA at 45°C in Petri dishes.

The disks, impregnated with the compounds (Table 1), were placed on the agar.

The petri dishes were incubated for 24-96h.

Antifungal activity was indicated by formation of inihibitions halos and the grouth on top of the disks.



2 Compound/ fungal liquide interaction assay

A 10^8 cfu/mL fungal suspension was obtained through dilutions and counting in a Neubauer chamber and ajusted to 10^5 cfu/mL by serial dilution.

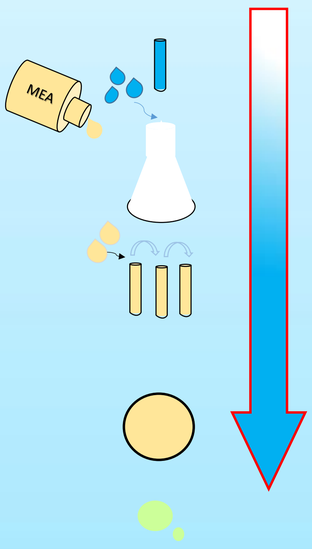
A mixture composed by malt extract, the fungus and the compounds (5 mM and 50 mM, each in duplicated) were incubated in flasks for 72h at 25 °c and 120 rpm.

Samples were taken after 24, 48 and 72h and a serial dilution were made.

The samples were plated, by incorporation in MEA.

Petri dishes were incubated for 24-72h.

The relative inhibition data is presented as quantity of cfu/mL against a control culture of the same fungi in the absence of the compounds.

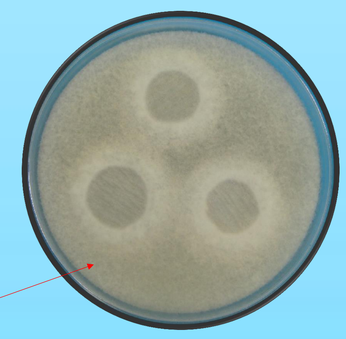


Results and discussion

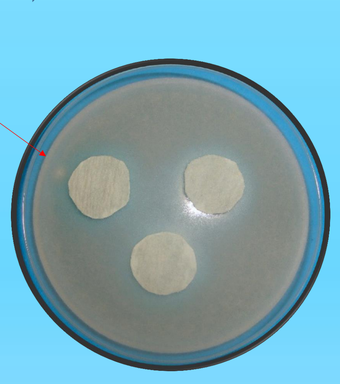
1 Antifungal diffusion assay

Table 2 - Results correspondent to antifungal activity against *F. oxysporum*, *A. niger*, *T. harzianum* and *Cladosporium sp* against all the coumarins using the paper disks diffusion assay.

		866	296	7F1	783
A		-	-	+/-	-
B		++++	+++	+	+
C	5 mM	+	-	-	-
		-	+		
D	5 mM	+	-	++	+/-
		-	+/-		-
E	50 mM	+	+/-	+	-
		+++	+/-	+	++
	5 mM	+	-		-
		+	+	-	+
		-	-	-	+
	0,5 mM	-	-	-	+/-
F	50 mM	+++	+	+	++
		+++	+	++	+
	5 mM	+	-	+/-	+/-
		+	+	++	+/-
		-	-		+
	0,5 mM	-	-	-	+/-
G	5 mM	+	+/-	+/-	-
		-	+	+	-
		+/-	-		+/-
H	5 mM	+	+/-	+/-	-
		+	+	-	-
		+/-	-		-
Control	0 mM	-	-	-	-
		-	-	-	-
		-	-	-	-
		-	-	-	-



The most active compounds were 3-Bromo-7-Methoxycoumarin (E) and 3-Bromo-6,7-Dimethoxycoumarin (F)



- 866 - *F. oxysporum*, 296 - *A. niger*, 7F1 - *Cladosporium sp* and 783 i-*T. harzianum*;
- Inhibitory activity of the compounds is classified as follows: - no inhibition, ± very low inhibition, + low inhibition, + + moderate inhibition, +++ high inhibition, + + + + complete inhibition.

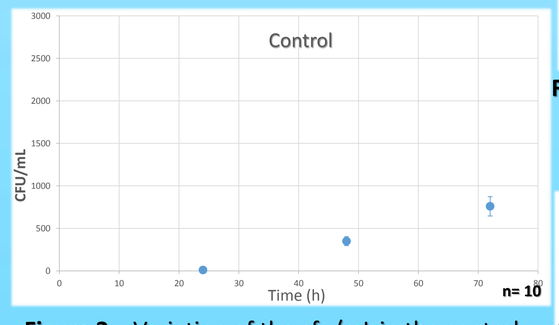


Figure 3 – Variation of the cfu/mL in the control flask, in the fungal/compound liquide interaction assay during 72 hours.

2 Antifungal interaction assay

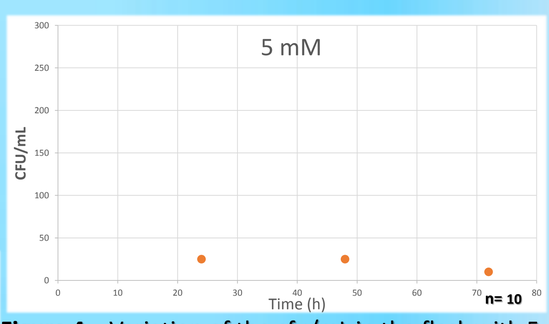


Figure 4 – Variation of the cfu/mL in the flask with 5 mM of 7M 3Br in the fungal/compound liquide interaction assay with *A. niger* during 72 hours.

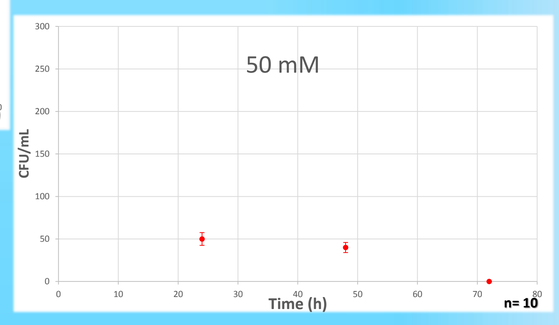


Figure 5 – Variation of the cfu/mL in the flask 50 mM of 7M 3Br in the fungal/compound liquide interaction assay with A. niger during 72 hours

Conclusion

The higher activity revealed by the 3-bromo-7-methoxycoumarin (**E**) suggests that the electron delocalization induced by the 7-methoxy group affects directly the 3-position, causing such differences in activity. The lowest concentration of the compound show anti-fungal activity at the lowest concentration in the first 24 hours and a total inhibition of growth after 72 hours of interaction at 50,0 mM.
Results encourage us the pursue more research with 3-bromo-7-methoxycoumarin and also 3-bromo-6,7-dimethoxycoumarin. The understanding of molecular mechanisms involved in the antifungal activity should also be pursued.

References

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