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Screening of Amino-acid derived Thioureas as Antimicrobials for the Primary Sector

Concepción González;[1] Zuleima González; [1] Natalia Expósito; [1] Javier García-Machado; [1] Cristina Giménez; [2] Alicia Boto. [1]

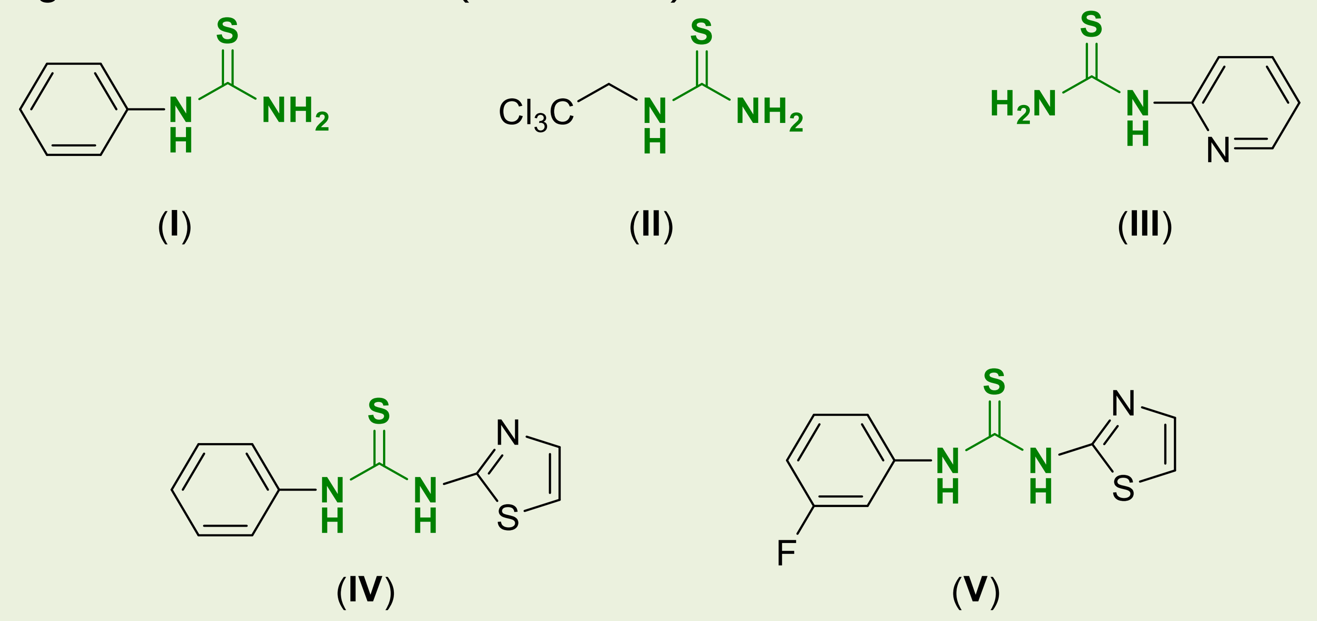
[1] *Instituto de Productos Naturales y Agrobiología del C.S.I.C. Avda. Astrofísico Fco. Sánchez 3, 38206 La Laguna, Tenerife, España..* [2] *UDI Fitopatología, Facultad de Ciencias, Universidad de La Laguna (ULL), ES- Aptdo. 456. 38200 La Laguna, Tenerife, Spain.*

Email: zuleima@ipna.csic.es

INTRODUCTION

In spite of the advances in pest and phytopathogen control using biological and physical agents, in the last fifty years control has relied primarily on the use of synthetic chemical pesticides. However, since the mid-20th century, the types of compounds have changed substantially to increase selectivity and decrease environmental impact. Since requirements for the authorisation of a new plant protection product are increasingly stringent, the search for new active compounds is an area of great interest. Among fungicides, both inorganic compounds (copper salts, sulphur) and organic products (dithiocarbamates, phthalimides, etc) are used.[1]

In this context, we can highlight derivatives containing **urea** or **thiourea** moieties. In particular, some examples have been described that are of particular interest in agriculture as they can affect insect or plant growth, but most importantly, they can act as fungicides or herbicides (Scheme 1).[2]



Scheme 1. Examples of thioureas derivatives used in agriculture as fungicide.

In our research on **new thioureas**, we have focused on the synthesis of **fluorinated thioureas derived from amino acids**. In this communication, we will present our preliminary results of the synthesis of these compounds and also the evaluation obtained against different phytopathogenic fungi (***Alternaria alternata*****,** ***Botrytis cinerea*** **y** ***Fusarium oxysporum***). The data obtained therefrom are used to perform a structure-activity relationship (SAR) study to determine the structural characteristics that could improve the selectivity and potency of these compounds.

SYNTHESIS OF THIOUREAS

First, a library of **40** different **ureas** and **thioureas** was prepared. For this purpose, different amino acids (protected as methyl ester or not) were reacted, in the presence of triethylamine (TEA), with the corresponding **(thio)isocyanate derivative reagent** (Figure 1). All the resulting products were crystalline solids and were achieved in moderate to good yields (60-90%). The one-step production of the products and the possibility to scale them up to grams is a real advantage for use in agriculture.

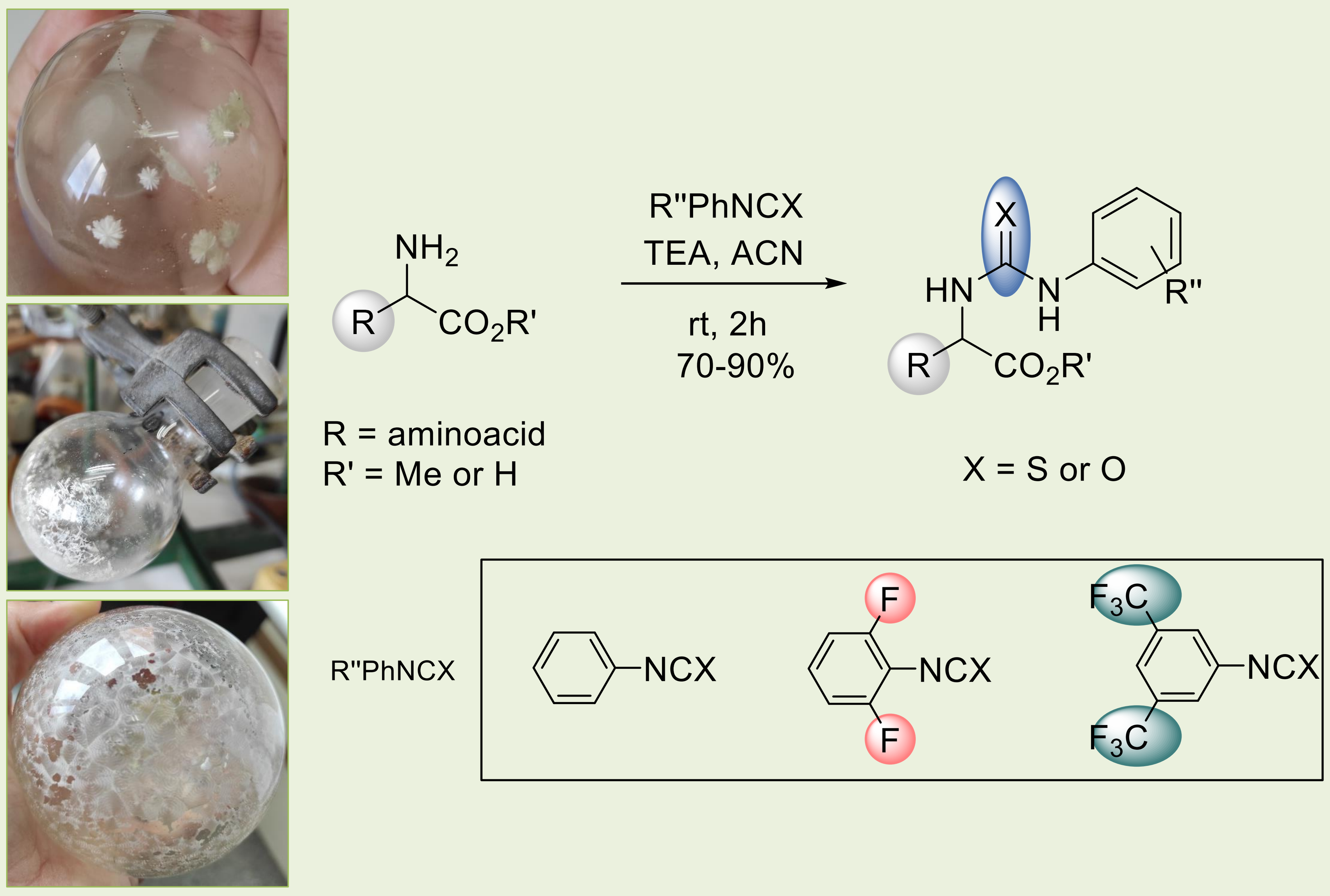


Figure 1. General synthetic scheme of the **ureas** and **thioureas** prepared for this study.

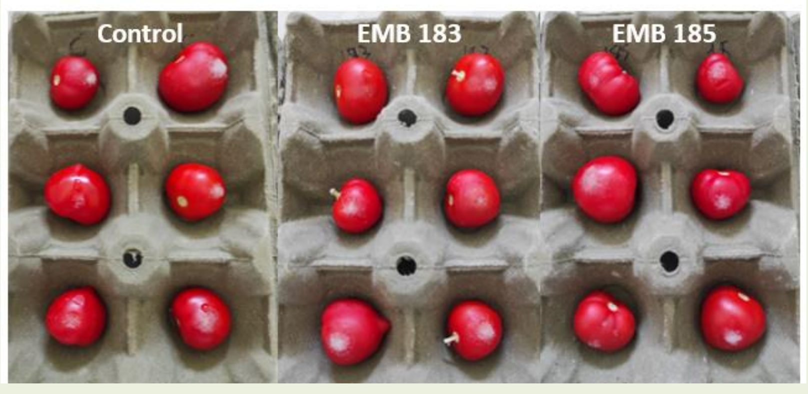
IN VITRO ASSAYS

For the *in vitro* studies, we used three different fungi (***A. alternata*****,** ***B. cinerea*** **y** ***F. oxysporum***). Once the compounds were evaluated, we selected those that, at a dose of 1 mg/mL, were able to inhibit 60% of the fungal growth. Of the 40 compounds, only 4 showed promise (**EMB 183**, **EMB 185**, **EMC 7B**, **EMC 23A**).

IN VIVO ASSAYS

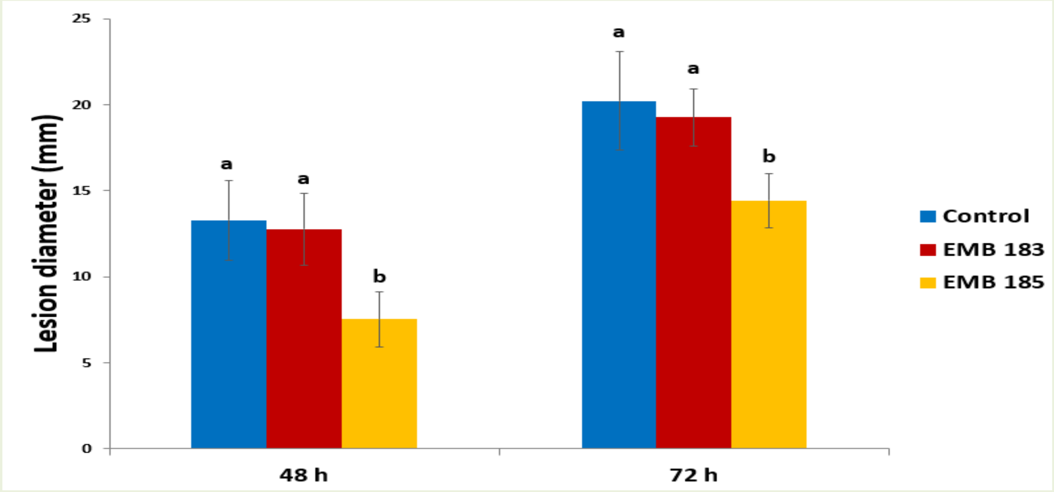
With the compounds selected above (**EMB 183**, **EMB 185**, **EMC 7B** and **EMC 23A**), *in vivo* assays with *B. cinerea* were performed on **leaves** of ***Vitis vinifera*** and on **fruits** of the **micro Tom *Solanum lycopersicum*** variety, which were grown in our greenhouse (Figure 2).

Figure 2. Growing micro Tom plants in our greenhouse.



To test the preventive effect of **EMB 183** and **EMB 185**, tomatoes were sprayed at a concentration of 1 mg/mL and infected by wounding with a suspension of $1x10^6$ conidia/mL of the fungus (Figure 3).

Figure 3. Infection of tomatoes with conidia.



As illustrated in Figure 4, only **EMB 185** showed a significant decrease in the diameter of the lesion caused by the fungus.

Figure 4. Lesion diameter caused by infection.

To test the preventive effect of the products **EMB 183**, **EMB 185**, **EMC 7B** and **EMC 23A**, **leaves** of ***V. vinifera*** were sprayed with a 1 mg/mL concentration solution of each product and infected with 5 mm diameter discs of the fungus (Figure 5).

Figure 5. Infection of **EMC 7B**-treated grapevine leaves with fungal discs.

In this test, it was observed that only **EMB 185** and **EMC 7B** showed a significant decrease in the diameter of the lesion caused by the fungus (Figure 6).

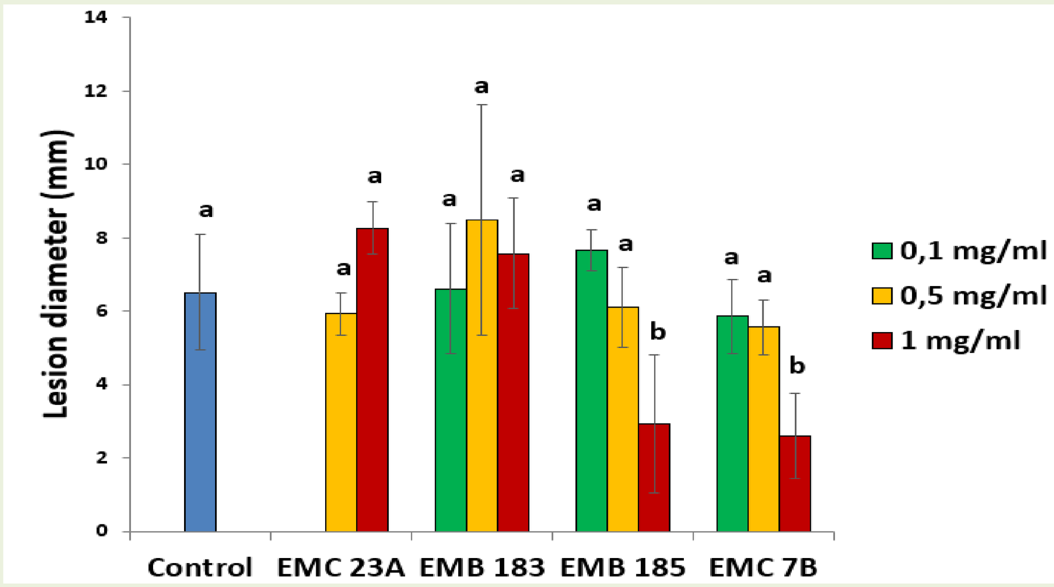


Figure 6. Diameter of lesion caused by infection.

CONCLUSION

- A first *in vitro* screening was performed with ***A. alternata*****,** ***B. cinerea*** **y** ***F. oxysporum***.
- Only 4 compounds exhibited possible antifungal activity and all of them turned to be fluorinated thioureas.
- *In vivo* tests with ***B. cinerea*** were performed on ***V. vinifera*** leaves and on fruits of the micro Tom ***S. lycopersicum*** variety.
- Only **EMB 185** and **EMC 7B** showed to be promised and are being assessed in further studies.

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2.- Shakeel , A.; Altaf, A. A.;*, Qureshi1, A. M.; Badshah, A. Journal of Drug Design and Medicinal Chemistry 2016; 2(1): 10-20.

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