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In vitro evaluation of the activity of thiosemicarbazone derivatives against mycotoxigenic fungi affecting cereals

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ABSTRACT

With a steadily increasing world population, a more efficient system of food production is of paramount importance. One of the major causes of food spoilage is the presence of fungal pathogens and the production and accumulation of mycotoxins. In the present work we report a study on the activity of a series of functionalized thiosemicarbazones (namely cuminaldehyde, *trans*-cinnamaldehyde, quinoline-2-carboxyaldehyde, 5-fluoroisatin thiosemicarbazone and 5-fluoroisatin N^4-methylthiosemicarbazone), as antifungal and anti-mycotoxin agents, against the two major genera of cereal mycotoxigenic fungi, i.e. *Fusarium* and *Aspergillus*. These thiosemicarbazones display different patterns of efficacy on fungal growth and on mycotoxin accumulation depending on the fungal species. Some of the molecules display a greater effect on mycotoxin synthesis than on fungal growth.

1. Introduction

Plant diseases are probably the greatest obstacle to the increase of global crop production and also one of the major factors limiting crop quality. Crop protection plays a key role in ensuring food security and, in turn, is directly related to food preservation, a key step to increasing both food supply and food safety. In fact about 40% of the food produced worldwide is lost or spoiled (Oerke and Dehne, 2004) and this not only reduces its availability, but also has an impact on global climate change by causing excess consumption of fresh water and fossil fuels.

One of the major causes of food spoilage is the presence of micro-organisms. In particular, fungal pathogens associated with various crop diseases can lead not only to significant production losses, but can also result in the production and accumulation of secondary metabolites – mycotoxins – hazardous to human and animal health. In cereals, the main mycotoxin producers belong to the genera *Aspergillus*, *Penicillium* and *Fusarium*. *Fusarium* are "field" fungi with worldwide distribution that can colonize the cereal plant during flowering causing Fusarium Head Blight (FHB), a destructive disease that leads to severe yield losses and to the accumulation of mycotoxins in the grains (Goswam and Kistler, 2005). Types A and B trichothecenes, and zearalenone are the most important toxic products that can occur individually or in combination. In European environments, the most common species in the FHB complex are *Fusarium graminearum*, *Fusarium culmorum*, *Fusarium sporotrichioides* and *Fusarium langsethiae*. As far as *Aspergillus* is concerned, a few species belonging to this genus (mainly *Aspergillus flavus* and *Aspergillus parasiticus*) may colonize various crops intended for human and animal nutrition. As these fungi are well known mycotoxin producers (mainly aflatoxins; AFs), they constitute a serious threat to humans and animals. In particular, *A. flavus* can be found in corn fields throughout the world and is responsible of severe economic losses for the farmers and the stakeholders of the post-harvest phase (Abbas et al., 2009; Williams, 2008; Wu and Khlangwiset, 2010). In fact, the AF risk for human health comes not only from direct consumption of crop materials, but may persist in processed products (milk, cheese, etc.) derived from animals fed with contaminated feed. Several and complementary strategies have been adopted to control FHB and AF, starting from the breeding of resistant cereal varieties, to the adoption of more suitable agronomic practices including the application of fungicides (for reviews see Abbas et al., 2009; Terzi et al., 2014). Currently, fungicides are required to increase food availability, to reduce food waste and to increase food safety (Cooper and Dobson, 2007). However the use of fungicides can lead, in the medium and long terms, to the evolution of populations of pathogens resistant to the

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antimicrobial agents used against them (Anderson, 2005; Ma and Michailides, 2005). Hence, the identification of new molecular targets becomes of paramount importance for novel strategies in crop protection.

A secondary but no less important issue that should be considered when testing new fungicides for their activity on growth inhibition concerns a possible contradictory and unwanted effect on mycotoxin biosynthesis; i.e. inhibition of mycelium growth resulting in an increase of toxin production (Schmidt-Heydt et al., 2013). In some cases, such as *A. flavus* colonization of maize crops, the prevalent economic damage emerges from the sanitary risk deriving from toxin contamination of the food matrix rather than to a negative effect on plant yield (van Egmond and Jonker, 2004).

Thiosemicarbazones, an important class of nitrogen and sulfur containing compounds, have received great attention because of their chemical and biological activities, such as antibacterial, antivirus, antiamoebic, and antitumor activities in human pharmacology (Pelosi, 2010). Numerous thiosemicarbazone derivatives are also active for the control of some human pathogens, however there are only a limited number of studies concerning their applications [see patents: CN 102219769 (2011) CN 103694155 (2014)]. Recently, data on the effect of some thiosemicarbazone metal complexes on a subset of plant pathogens were published (Tyagi et al., 2014); however the study was mainly aimed at characterizing the coordination compounds and investigating the influence of the metal on the features of the compounds, and a very limited part of the work was dedicated to mycological studies.

The aim of the present work was therefore to evaluate the potential of a panel of thiosemicarbazones, differing in their functionality, for crop protection and food spoilage control, with a particular focus on the biological activity of these compounds as antifungal and anti-mycotoxin agents against the two major genera of cereal mycotoxigenic fungi, i.e. *Fusarium* and *Aspergillus*.

2. Material and methods

2.1. Thiosemicarbazone synthesis

Fig. 1 depicts the synthesized thiosemicarbazones for which the acronyms reported in parentheses are used: cuminaldehyde thiosemicarbazone (**Htcum**), *trans*-cinnamaldehyde thiosemicarbazone (**Htcin**), quinoline-2-carboxyaldehyde thiosemicarbazone (**Htisq**), 5-fluoroisatin N^4-methylthiosemicarbazone (**HtmeFis**) and 5-fluoroisatin thiosemicarbazone (**HtFis**). All reagents used in the syntheses were purchased from Aldrich. C, H, and N analyses were obtained with a Carlo-Erba 1108 instrument. IR spectra were recorded using KBr pellets on a Nicolet 5PC FTIR spectrophotometer, or using the compounds directly on the ATR accessory in the 4000–400 cm^{-1} range. The relative intensity of reported FT IR signals are defined as s = strong, br = broad, m = medium, and w = weak. ^1H NMR spectra were recorded on a Bruker Avance spectrometer at 300 MHz with TMS as the internal reference. The splitting of proton resonances in the reported ^1H NMR spectra is defined as s = singlet, br s = broad singlet, d = doublet, t = triplet, and m = multiplet. Melting points were determined with a Gallenkamp instrument (Weiss-Gallenkamp). UV measurements were performed on a Varian UV–vis Cary 50 spectrometer with quartz cuvettes. The ligands were prepared following a modified procedure based on the following references: Bisceglie et al. (2014); Chiyanzu et al. (2003); Karali et al. (2007); Yi et al. (2011). **Htcum** and **Htcin** were synthesized by respectively adding 0.39 mL (2.3 mmol) of cuminaldehyde (*p*-isopropyl benzaldehyde) or 0.29 mL (2.3 mmol) of *trans*-cinnamaldehyde (3-phenyl-2-propenal) to 20 mL of a hot stirred ethanolic solution of thiosemicarbazide (0.21 g, 2.3 mmol). The mixture was then refluxed for 7–9 h and left cooling down to room temperature. **Htisq** was obtained by dissolving thiosemicarbazide (337.6 mg, 3.7 mmol) in 80 mL of methanol at room temperature under stirring and adding an equimolar amount of quinoline-2-carboxaldehyde (582.1 mg, 3.7 mmol). After adding a few drops of glacial acetic acid to catalyze the reaction, the solution became clear and its color turned to orange. This reaction mixture was kept under stirring and placed in an ice bath and left standing for 20 h. The white precipitate was filtered on a Buchner funnel and washed using ethanol. **HtmeFis** was synthesized by dissolving, in about 70 mL of methanol, 350.6 mg (3.3 mmol) of 4-methyl-3-thiosemicarbazide together with an equimolar amount of 5-fluoro-isatin (389.6 mg). Similarly, **HtFis** was obtained by reacting 110.6 mg (1.1 mmol) of thiosemicarbazide with an equimolar amount of 5-fluoro-isatin (200.3 mg) in about 60 mL of methanol. To the resulting orange solutions, few drops of glacial acetic acid were added and the resulting mixture was heated to reflux temperature under magnetic stirring for 24 h. A yellow powder was isolated by filtration using a Buchner funnel and washed 3 times with methanol.

2.1.1. Cuminaldehyde thiosemicarbazone (*Htcum*)

Yield: 86%. Mp: 144 °C. FT-IR (KBr, cm^{-1}) 3411, m, 3280, m, ν (NH); 3013, m, ν ($CH_{aromatic}$); 2957, mw, ν ($CH_{aliphatic}$), 1586, s, ν (C═N); 820,

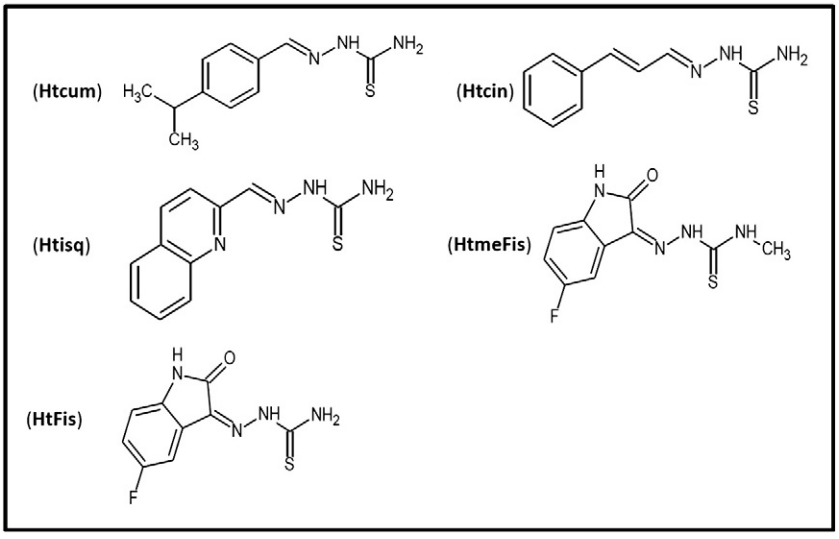

Fig. 1. Structures of the synthesized thiosemicarbazones. (**Htcum**) cuminaldehyde thiosemicarbazone; (**Htcin**) *trans*-cinnamaldehyde thiosemicarbazone; (**Htisq**) quinoline-2-carboxyaldehyde thiosemicarbazone; (**HtmeFis**) 5-fluoroisatin N^4-methylthiosemicarbazone; (**HtFis**) 5-fluoroisatin thiosemicarbazone.