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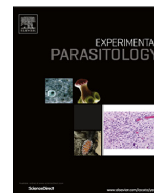

# Activity of chalcones derived from 2,4,5-trimethoxybenzaldehyde against *Meloidogyne exigua* and *in silico* interaction of one chalcone with a putative caffeic acid 3-*O*-methyltransferase from *Meloidogyne incognita*

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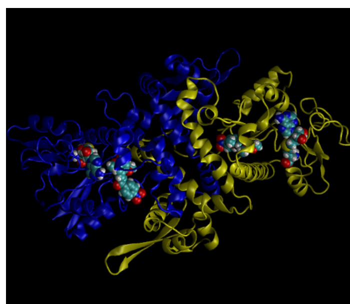
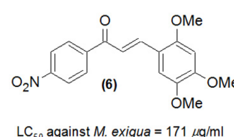
## HIGHLIGHTS

- Chalcones synthesized by aldolic condensations were active against *M. exigua* J2.
- One chalcone (**6**) reduced the population of *M. exigua* in coffee plants.
- Caffeic acid 3-*O*-methyltransferase was identified in the genome of *M. incognita*.
- *In silico* studies showed that chalcone **6** inhibits that enzyme.

## GRAPHICAL ABSTRACT






$LC_{50}$ against *M. exigua* = 171 μg/ml

Kd of the complex **6** - caffeic acid 3-*O*-methyltransferase = 3.1 x $10^{-9}$ mol/l

## ARTICLE INFO

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Protein modeling
Chalcone

## ABSTRACT

*Meloidogyne exigua* is a parasitic nematode of plants that causes great losses to coffee farmers. In an effort to develop parasitic controls, 154 chalcones were synthesized and screened for activity against this nematode. The best results were obtained with (2*E*)-1-(4′-nitrophenyl)-3-(2,4,5-trimethoxyphenyl)prop-2-en-1-one (**6**) with a 50% lethal concentration ($LC_{50}$) of 171 μg/ml against *M. exigua* second-stage juveniles, in comparison to the commercially-available nematicide carbofuran which had an $LC_{50}$ of 260 μg/ml under the same conditions. When coffee plants were used, **6** reduced the nematode population to ~50% of that observed in control plants. To investigate the mechanism of action of **6**, an *in silico* study was carried out, which indicated that **6** may act against *M. exigua* through inhibition of a putative caffeic acid 3-*O*-methyltransferase homodimer, the amino acid sequence of which was determined by examining the genome of *Meloidogyne incognita*.

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## 1. Introduction

Plant-parasitic nematodes are responsible for the loss of ~10% of all global agricultural production (Zacheo, 1993). An example is coffee, whose production can be hugely affected by *Meloidogyne*

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*exigua* Goeldi (Souza, 2008). Once this nematode is established in coffee plantations, the most efficient methods of control are based on the use of synthetic nematicides such as aldicarb [(5*E*)-7,7-dimethyl-4-oxa-8-thia-2,5-diazanon-5-en-3-one], carbofuran (2,2-dimethyl-2,3-dihydro-1-benzofuran-7-yl methylcarbamate), and terbufos {*S*-[(*tert*-butylsulfanyl)methyl] *O*,*O*-diethyl phosphorothioate}, which increase the cost of production and may adversely affect humans and the environment (Chitwood, 2002). Consequently, alternatives to nematode control have been sought among natural products and analogues, yielding promising chemicals such as chalcones, which are flavonoid precursors in plants (Dewick, 2002) that have activity against the cist nematodes *Globodera rostochiensis* Wollenweber and *Globodera pallida* Stone (González and Braun, 1998). Nevertheless, studies on methods of nematode control using chalcones are scarce.

Chalcones can be prepared in a number of ways (Sebti et al., 2001; Eddarir et al., 2003; Petrov et al., 2008), the simplest being aldolic condensation between benzaldehydes and acetophenones (McMurry, 2000). This method allows for the rapid synthesis of several chalcones using common inexpensive reagents and was used to prepare a library of 154 synthetic chalcones with different substitutions that were subsequently screened for activity against *M. exigua*. The mechanism of action for these chemical substances was studied *in silico* for the interaction of chalcone **6** [(2*E*)-1-(4′-nitrophenyl)-3-(2,4,5-trimethoxyphenyl)prop-2-en-1-one] with a putative caffeic acid 3-*O*-methyltransferase from *Meloidogyne incognita* (Kofoid and White) Chitwood, which is similar to *M. exigua* and may also cause damage to coffee plants (Souza, 2008).

## 2. Materials and methods

### 2.1. Chemicals and general synthetic procedure

Unless otherwise noted, solvents and reagents were used as received from commercial suppliers (Sigma–Aldrich, Merck, Fluka and Vetec). The chalcones were prepared by aldolic condensation between substituted benzaldehydes (1 mmol) and the appropriate acetophenones (1 mmol) in methanol (20 ml) containing potassium hydroxide (0.5 g/ml) at room temperature with magnetic stirring for 24 h after which synthesized compounds were precipitated with water and 0.1 g/ml hydrochloric acid. The purity of each synthesized chalcone was evaluated by thin layer chromatography (TLC) using aluminum plates pre-coated with silica gel (200 μm in thickness) using several solvent systems with different polarities. Compounds were visualized with ultraviolet light (wavelength = 254 and 360 nm) or using sulfuric anisaldehyde solution followed by heat as the developing agent. Compounds were recrystallized from hot ethanol to obtain pure chalcones at yields between 61% and 93%. Chalcones **1**–**6** and **10** of the active series, derived from 2,4,5-trimethoxybenzaldehyde, have been previously described (Patil et al., 2006; Borchhardt et al., 2010; Wang et al., 2010; Kumar et al., 2010; Ávila et al., 2008; Rosli et al., 2006; Kharazmi et al., 1999) while **7**–**9** and **11** are novel compounds. The novel chalcones were soluble in dimethyl sulfoxide, acetone, ethyl acetate and chloroform. Their structures were confirmed through melting point (mp) analysis and nuclear magnetic resonance (NMR) spectroscopy. For the unpublished chalcones, elemental (CHN) analysis was also carried out and percentages of C, H and N were in agreement with the molecular formulas of the compounds (within ±0.4% of theoretical values for C).

### 2.2. Chemical characterization of the synthesized compounds

Melting points were determined on a Microquímica MGAPF-301 apparatus and were uncorrected. The CHN analysis was carried out using a CHNS EA 1110 analyzer. $^1$H and $^{13}$C NMR spectra were recorded on a Bruker AC-200F (200 MHz) or a Varian Oxford AS-400 (400 MHz) instrument, using tetramethylsilane as the internal standard and deuterated chloroform ($CDCl_3$) as solvent. $^1$H NMR spectra revealed that all the structures were geometrically pure *E* isomers ($J_{H\alpha-H\beta}$ = 16.0 Hz). The chemical data of the compounds derived from 2,4,5-trimethoxybenzaldehyde (**1**–**11**) are presented in the Supplemental material.

### 2.3. In vitro screening for activity against M. exigua

Nematode eggs were extracted from infected coffee roots (*Coffea arabica* L.) as described by Hussey and Barker (1973) and modified by Boneti and Ferraz (1981). The eggs were allowed to hatch and second-stage nematode juveniles (J2) were collected. Only two-day old J2 were used for the *in vitro* assays carried as described by Amaral et al. (2002). Briefly, an aqueous suspension containing approximately 25 J2 (in 20 μl) and 100 μl of the sample in aqueous 0.01 g/ml Tween 80 were added into each 300 μl well of a polypropylene 96-well plate, resulting in a final concentration of 500 μg/ml for each chalcone. After 24 h at 28 °C, one drop of a freshly prepared aqueous 1.0 mol/l sodium hydroxide solution was added and any J2 that moved within 3 min were considered to be alive, whereas nematodes not responding to the sodium hydroxide were considered dead. This experiment was performed with four replicates per treatment, using 0.01 g/ml Tween 80 and the commercial nematicide aldicarb at 50 μg/ml as negative and positive controls, respectively. For aldicarb solution preparation, 8.6 g of Temik 150 (150 g of aldicarb/kg; Rhône-Poulenc AgroBrasil Ltda.) was dissolved in water, filtered through filter paper and the resulting solution diluted with water to the desired concentration. All J2 mortality values were converted to percentages before analysis of variance (ANOVA), after which, means were separated according to the Scott and Knott (1974) test ($P \leqslant 0.05$). Statistical analyses were done using SISVAR 5.1 software (Sistema para análises Estatísticas, UFLA, Lavras, 2006).

### 2.4. Determination of 50% lethal concentration ($LC_{50}$)

Solutions of carbofuran (Sigma–Aldrich, 98%), aldicarb and chalcone **6** at different concentrations were used for the *in vitro* assay with *M. exigua* J2 as described above (0.01 g/ml Tween 80 solution was control). The final concentrations were 224, 244, 265 and 296 μg/ml carbofuran; 50, 40, 30, 20 and 10 μg/ml aldicarb; and 120, 150, 180, 210 and 240 μg/ml chalcone **6**. Average mortality values for J2 were converted to percentages, corrected {corrected value = 100 [(value − control value)/100 − control value]} and submitted to probit analyses by the POLO-PC software (LeOra Software, 1987).

### 2.5. Assay with M. exigua inoculated coffee plants

A mixture of soil and sand (1:1 by volume; substrate) was disinfested with methyl bromide (150 ml/m$^3$), poured into 3 l pots and a six-month old coffee plant (*C. arabica* L. cv. Catucaí Amarelo) planted in each pot. After 20 days in a greenhouse, each plant was inoculated with approximately 10,000 *M. exigua* eggs suspended in 10 ml of water through four equidistant cylindrical holes 1.0 cm wide and 4.0 cm long in the substrate around the plant. Immediately after each inoculation, treatments were added through the same holes: 100 ml of an aqueous 0.01 g/ml Tween 80 solution containing chalcone **6** (1796, 3591 or 7182 μg/ml) or aldicarb (500 μg/ml) were used. After 90 days in a greenhouse, the aerial portion of the plant was removed, the roots were gently washed with water, weighted and submitted for gall counting. Then nematode eggs were extracted from roots as described elsewhere

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