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Activity of caffeic acid derivatives against *Candida albicans* biofilm

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ABSTRACT

The aim of this study was to evaluate the caffeic acid (**1**) and ester derivatives (**2**–**10**) against *Candida albicans* biofilm and to investigate whether these compounds are able to inhibit the biofilm formation or destroy pre-formed biofilm.

Caffeic acid ester **7**, cinnamic acid ester **8** and 3,4-dihydroxybenzoic acid ester **10** are more active than fluconazole, used as reference drug, both on biofilm in formation with MIC_{50} values of 32, 32 and 16 μg/mL, respectively, and in the early stage of biofilm formation (4 h) with MIC_{50} values of 64, 32 and 64 μg/mL, respectively. These esters result also more active than fluconazole on mature biofilm (24 h), especially **8** and **10** with MIC_{50} values of 64 μg/mL.

Candida species are opportunistic pathogens that can cause a wide variety of infections and they are the main agents responsible for nosocomial fungal infections. *Candida* spp. can form readily biofilm on indwelling medical devices and mucosal tissues. This biofilm turns out to be an infectious reservoir difficult to eradicate, and it causes device-associated infections with high mortality.[1,2]

The most prevalent fungal biofilm-forming pathogen is *Candida albicans*, which cause both superficial and systemic infections. *C. albicans* is able to colonize and form biofilms on devices such as shunts, stents, endotracheal tubes and various types of catheters.[3] Indeed, it has been reported that infections caused by biofilm-forming *C. albicans* were significantly correlated with increased mortality.[4]

The early stage of *C. albicans* biofilm formation is characterized by the adhesion of single cells to the substratum, that was followed by the formation of an intricate network of hyphae and the beginning of a dense structure. Changes in the transcriptome begin within 30 min of contact with the substrate. Some of these changes are initiated early and maintained throughout the process; other changes are typical of the earliest stages of biofilm formation.[5]

Among the phenotypic alterations displayed by cells in biofilms, the most relevant from the clinical point of view is the increased resistance to antifungal treatments.[6,7] Compared to their planktonic counterparts, biofilm cells exhibit up to a 1000-fold increased resistance.

C. albicans biofilms have been reported to be resistant to a variety of clinical antifungal agents, including fluconazole,[8] a well-tolerated antifungal drug, commonly used in the treatment of candidiasis. Among the available antifungal agents, only echinocandins and amphotericin B lipid formulations have shown consistent activity against *Candida* biofilms. Unfortunately, amphotericin B can cause severe nephrotoxicity to the host and echinocandins are highly expensive to be used routinely.[9] Therefore, in this scenario, there is an urgent need for low-cost compounds with a better efficacy and low side effects.

Several molecules were reported in literature to inhibit or to prevent the *C. albicans* biofilm formation. Many of these compounds are characterized by phenylethenyl moieties, such as 4-hydroxycordoin and methyl cinnamate;[10,11] among these molecules, caffeic acid phenethyl ester (CAPE) is able to inhibit both *Candida* filamentation and biofilm formation (Chart 1).[12]

We have decided to test caffeic acid (**1**) and the ester derivatives (**2**–**10**) against *C. albicans* planktonic and biofilm cells, in order to evaluate the effects on anti-biofilm activity of structural modification on the alcoholic and carboxylic moieties. The assessment of anti-biofilm activity of these compounds may allow to understand

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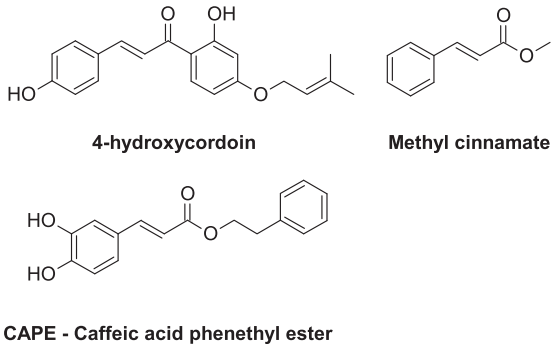
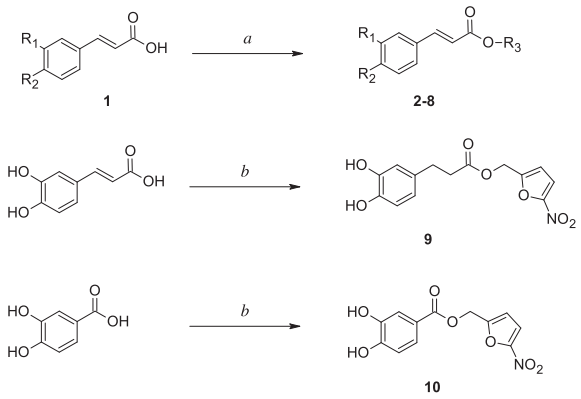


Chart 1. Phenylethenyl derivatives active on *C. albicans* biofilm.



Scheme 1. Synthetic procedures. (a) (Compounds **1**–**5**): H_2SO_4, R_3-OH, reflux; (compounds **6** and **7**): (i) NaOH, DMF, rt, 20 min; (ii) 4-(bromomethyl)-2-fluoro-1-methoxybenzene (for **6**), 2-(bromomethyl)-5-nitrofuran (for **7**), rt, 50 h. (Compound **8**) (i) Cs_2CO_3, DMF, rt, 20 min; (ii) 2-(bromomethyl)-5-nitrofuran, 12 h, rt. (b) (i) NaOH, DMF, rt, 20 min, (ii) 2-(bromomethyl)-5-nitrofuran, 20 h, rt; (iii) 2 h, 50 °C.

the real importance of phenylethenyl group trying to delineate a basic SAR rules.

We have synthesized different esters modifying the alcoholic function with simple small alkyl chains (**2**–**5**), with small hydrophobic aromatic moiety (**6**) and nitro-furan group (**7**) that was chosen on the basis of its known antibacterial properties.[13]

Furthermore, we have also modified the acylic moiety removing the hydroxyl groups (**8**), saturating (**9**) or removing the ethenyl double bond (**10**) (Chart 2).

The synthetic procedures were described in Scheme 1. Caffeic acid esters (**2**–**5**) have been synthesized by Fisher esterification starting from commercially available caffeic acid (**1**) as described in the literature.[14] The derivatives **6** and **7** were prepared via the direct carboxylate oxygen alkylation of the caffeic acid, respectively, by 2-(bromomethyl)-5-nitrofuran and 4-(bromomethyl)-2-fluoro-1-methoxybenzene,[15] according to the methodology reported by Prasad et al.[16]

Similarly, compound **8** was prepared by O-alkylation of cinnamic acid with 2-(bromomethyl)-5-nitrofuran using Cs_2CO_3 as basic catalyst,[17] following the procedure described by Parrish et al.[18] and the esters **9** and **10** have been synthesized by direct O-alkylation of 3,4-dihydroxybenzylacetic acid and 3,4-dihydroxybenzoic acid with 2-(bromomethyl)-5-nitrofuran in presence of aqueous 0.4 M NaOH,[19] as described by Brewster et al.[20]

The analytical and spectroscopic data of new synthesized compounds **6**–**10** are reported in Table S.1 in the Supplementary material and are in agreement with the proposed structures.

Caffeic acid (**1**) and the ester derivatives **2**–**10** have been tested, as described below, against planktonically grown *C. albicans* cells and against *C. albicans* in formation biofilm, and early stage (4 h) and mature (24 h) *C. albicans* biofilms.

For the in vitro antifungal experiments *C. albicans* ATCC 10231 purchased by the American Type Culture Collection (ATCC, Rockville, MD, USA) were used. This strain is sensible to fluconazole on planktonic cells (0.5 μg/mL) and it is resistant in the different phases of biofilm formation. Antifungal activity of **1**–**10** and fluconazole (Table 1) against *C. albicans* was evaluated in accordance with the CLSI M27–A3 broth microdilution method.[21] The minimal inhibitory concentration (MIC) was calculated and expressed as the lowest drug concentration at which a significant decrease in turbidity (>50%) was detected in comparison with the control in the absence of drug.

The effect of **1**–**10** and fluconazole on biofilm was evaluated as described by Pierce et al. The anti-biofilm activity was evaluated and expressed as the lowest drug concentration at which a 50% decrease in absorbance was detected in comparison with the level for the biofilms formed in the absence of drug.[22]

The cytotoxicity of compounds **7**–**10** (Table 1) was evaluated on human pulmonary mucoepidermoid carcinoma cell line (NCI-H292) obtained from the ATCC (Rockville, MD, USA) using a

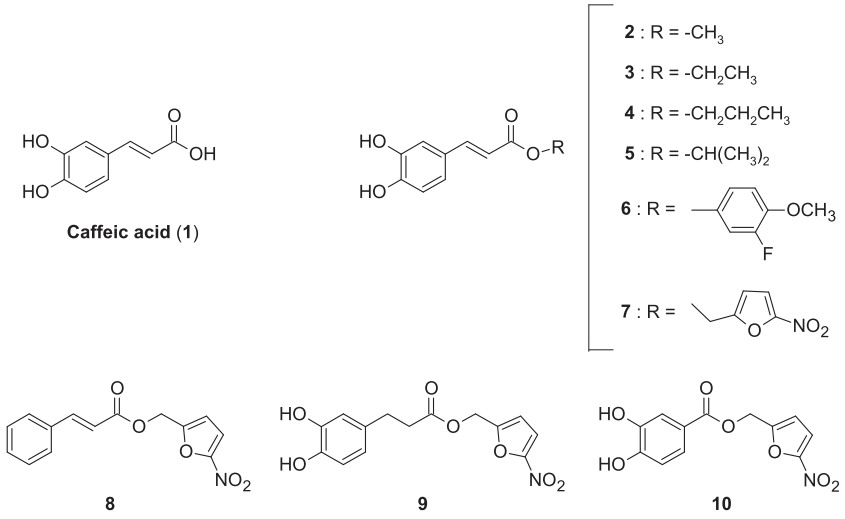


Chart 2. Synthesized compounds.