Preynlated Trans-Cinnamic Esters and Ethers against Clinical Fusarium spp.: Repositioning of Natural Compounds in Antimicrobial Discovery

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Abstract: Onychomycosis is a common nail infection mainly caused by species belonging to the F. oxysporum, F. solani, and F. fujikuroi species complexes. The aim of this study was to evaluate the in vitro susceptibility of six representative strains of clinically relevant Fusarium spp. toward a set of natural-occurring hydroxycinamatic acids and their derivatives with the purpose to develop naturally occurring products in order to cope with emerging resistance phenomena. By introducing a preynlated chain at one of the hydroxy groups of trans-cinnamic acids 1–3, ten preynlated derivatives (coded 4–13) were preliminarily investigated in solid Fusarium minimal medium (FMM). Minimal inhibitory concentration (MIC) and lethal dose 50 (LD50) values were then determined in liquid FMM for the most active selected antifungal p-coumaric acid 3,3′-dimethyl allyl ester 13, in comparison with the conventional fungicides terbinafine (TRB) and amphotericin B (AmB), through the quantification of the fungal growth. Significant growth inhibition was observed for preynlated derivatives 4–13, evidencing ester 13 as the most active. This compound presented MIC and LD50 values (62–250 µM and 7.8–125 µM, respectively) comparable to those determined for TRB and AmB in the majority of the tested pathogenic strains. The position and size of the preynlated chain and the presence of a free phenol OH group appear crucial for the antifungal activity. This work represents the first report on the activity of preynlated cinnamic esters and ethers against clinical Fusarium spp. and opens new avenues in the development of alternative antifungal compounds based on a drug repositioning strategy.

Keywords: onychomycosis; mycoses; Fusarium spp.; drug development; antifungal activity; phenolic inhibitors; hydroxycinamic acid derivatives; p-coumaric acid 3,3′-dimethyl allyl ester

1. Introduction

Phenolics are among the most desirable food components because of their excellent antioxidant activity and nutraceutical properties. They also find wide-ranging application in medicine and agriculture in virtue of their antimicrobial, anti-inflammatory, and antitumoral activities [1,2]. Among the phenolic compounds, cinnamic acids are a group of aromatic carboxylic acids formed through the biochemical route of shikimate pathway, leading to the synthesis of lignin, the polymeric material that provides mechanical support to the plant cell wall [3,4]. p-Coumaric acid 1, caffeic acid 2, and ferulic acid 3 are the most common cinnamic acids, consisting of a trans-α, β-unsaturated carboxylic chain bonded to a phenol, catechol, and guaiacyl unit, respectively. They possess three distinctive
structural motifs that may possibly contribute to the free radical scavenging capability of these compounds (Figure 1).

![Figure 1. Chemical structures of p-coumaric acid 1, caffic acid 2, and ferulic acid 3.](image)

The presence of an electron donating group on the benzene rings provides the additional property of terminating free radical chain reaction. The carboxylic acid group with a conjugated C-C double bond provides additional quenching sites for free radicals. These features of cinnamic acids are reflected in many biological processes. Cinnamic acids, the main constituents of plant defense, prevent the effects of reactive oxygen species (ROS) formed during fungal infection [5]. The carboxylic acid group of cinnamic acid can act as an anchor by which the compound binds to the lipid bilayer, providing some protection against lipid peroxidation, a process spread out across mammal and human cells [6].

Cinnamic acids 1–3 are widely distributed in fruits (e.g., apple, pear, grape, orange, tomato, and berries), vegetables (e.g., bean, potato, and onion), and cereals (e.g., maize, oat, and wheat bran) [7]. They occupy a key place as intermediates in the synthesis of pharmaceuticals, dyes, flavorings, cosmetics, thermoplastics, and materials [8,9]. Because of their high-promoting health capacities and commercial value and given the availability of cinnamic acids 1–3 in different plants, extracting processes from biomass are also well studied even though the main accessibility of these compounds comes from synthetic or microbial processes [10,11].

Drug repositioning implies the identification of novel biological targets for natural-occurring compounds that may find application in fields where safety and efficiency are still lacking. One approach relies on slight structural modification of the natural compound, aiming to enhance its biological activity toward a specific target. When the structural modification of the compound concerns the introduction of a natural unit to the molecule framework, the final compound acquires a natural-like feature. Often, such slight structural modifications are devoted to improving bioavailability and selectivity of the parent compound [12–16].

Onychomycosis is a chronic fungal nail infection mainly caused by Fusarium spp., particularly those belonging to three specie complexes: F. oxysporum (FOSC), F. solani (FSSC), and F. fujikuroi species complex (FFSC) [17–21]. F. oxysporum Schlechtend. emend. Snyder & Hansen; F. solani (Mart.) Sacc.; F. petrophilum (Q.T. Chen & X.H. Fu) Geiser, O’Donnell, D.P.G. Short, & N. Zhang; F. keratoplasticum Geiser, O’Donnell, D.P.G. Short, & Ning Zhang; F. falciforme (Carrión); and F. verticilloides (Saccardo) Nirenberg are the most representative species responsible for onychomycosis; moreover, they also infect skin and hair and are considered as emerging pathogens from superficial mycoses as dermatomycoses. Fusarium spp. are increasingly reported among the world population. Besides dermatomycoses or onychomycoses, they are responsible for disseminated infections, particularly in patients undergoing cancer therapy or those affected by immunological deficiency [22–24].

Systemic antifungals are the most effective treatment, with meta-analyses showing mycotic cure rates of 76% for terbinafine, 63% for itraconazole with pulse dosing, 59% for itraconazole with continuous dosing, and 48% for fluconazole [25,26]. The use of these agents is discouraged in patients suffering from liver, renal, or heart disease, and in those receiving medications with which there may be significant drug–drug interactions [27]. Terbinafine (TRB) belongs to the allylamine class of synthetic antymycotic agents, and inhibits the squalene epoxidase, a key enzyme involved in the early phase of the ergosterol biosynthetic pathway [28]. Amphotericin B (AmB) is a broad-spectrum antifungal agent belonging to the polyene class; its mechanism of action targets membrane function by
forming channels in the fungal cell membrane, hence allowing ions and organic compounds of the cytoplasm to escape [29].

Susceptibility to fungicides among different Fusarium spp. may vary greatly, and clinical Fusaria showing multiple resistance to most applied antifungal drugs are increasingly being reported [30–32]. Numerous factors have been cited to explain the lack of response to therapy, such as nonadherence to treatment, incorrect diagnosis, or advanced disease [25]. Additionally, antymycotic prophylaxis in high-risk patients may enhance selective pressure, which favors multidrug-resistant fungi, including Fusaria [33]. This urges a massive investment in the development of novel agents to treat emerging and resistant fungi [34].

Considering the antimicrobial activity of cinnamic acids 1–3 [35], their low toxicity, and large market availability, the aim of this work was to investigate these compounds against a selection of representative strains belonging to the F. oxysporum (FOSC), F. solani (FSSC), and F. fujikuroi (FFSC) species complexes; namely, F. oxysporum, F. solani F. keratoplasticum, and F. verticilloides.

Aiming to improve the bioavailability and to enhance antimicrobial activity, we transformed cinnamic acids 1–3 in esters and ethers using short, medium, and long prenylated chains as alcoholic unit. The efficiency of prenylated phenols in crossing bacterial and fungal membranes [36], as well as their role in exerting antimicrobial activity [37], are generally acknowledged. O-prenylated phenols are secondary metabolites of plants. Even though they have been considered for years as biosynthetic intermediates of the most widespread C-prenylated derivatives, recently, O-prenylated chains are assuming a key role in the bioactivity of molecules into which they are embedded [38,39]. We thus predicted that the preparation of prenylated ethers and prenylated esters of cinnamic acids 1–3 (Figure 2) could offer an alternative to the conventional antifungal drugs used against clinical Fusaria, enhancing the antymycotic effect of the parent acid.

![Chemical structure of the tested compounds numbered from 1 to 13.](image)

**Figure 2.** Chemical structure of the tested compounds numbered from 1 to 13.

An in vitro assay of clinical isolates of Fusarium spp. grown on solid Fusarium minimal medium (FMM) amended with compounds 1–13 was carried out and a structure–activity relationship was described. Minimal inhibitory concentration (MIC) and lethal dose 50 (LD50) values were then determined in liquid FMM for the most active selected antifungal compound, in comparison with the conventional fungicides terbinafine (TRB) and amphotericin B (AmB). The presence of a O-prenylated chain in natural occurring cinnamic acids
may share a different mode of action compared to conventional antifungal drugs and offers a successful example of drug repositioning.

2. Results
2.1. Chemistry

Prenylated esters 4–7 and 9 and prenylated ethers 10–13 were prepared, starting from the corresponding cinnamic acid 1–3 and, according to the reaction, allyl, 3,3′-dimethyl allyl (prenyl), geranyl, and citronellyl bromide or alcohol. Because of the contemporary presence of two hydroxyl groups in the starting hydroxy cinnamic acid, different synthetic approaches were applied when ether or ester was the final product.

In order to functionalize selectively the phenolic-OH group with a prenylated chain, esterification of the carboxylic group was mandatory (Scheme 1).

Scheme 1. Synthesis of hydroxyl cinnamic ethers 4–7 and 9 and esters 10–13 and 14–16.

Ethers 4–7 and 9 were obtained by the Williamson reaction, starting from the corresponding methyl ester and the appropriate alkyl bromide under basic conditions and further ester hydrolysis. Yields varied in the range of 53 and 85%. Methyl esterification of acids 1–3 was carried out under acidic conditions under microwave treatment.

For compound 5, the selectivity of etherification reaction at the p-phenolic-OH was confirmed by Nuclear Overhauser Effect Spectroscopy-NMR (NOESY) experiment and by comparison of NMR spectra with the corresponding methyl ester reported in the literature (see Materials and Methods for references).

Esters 10–13 were prepared with yields ranging between 37 and 47%, starting from the corresponding parent acid (i.e., 4-hydroxy cinnamic acid 1, caffeic acid 2, and ferulic acid 3) via activation of the carboxylic group with ethyl chloroformate and triethylamine and further addition of the appropriate prenylated alcohol (Scheme 1) and hydrolysis of the phenyl ethyl carbonate.

Compound 8 was obtained via Fischer esterification of acid 2 using allyl alcohol as solvent under acidic conditions as reported in the literature. Compound 13 was also obtained in 87% yield by enzymatic transesterification of the corresponding ethyl ester, in turn achieved by the microwave method, as well as 3,3′-dimethyl allyl alcohol, in mild conditions.

The purity of all new compounds was judged to be >98% by 1H-NMR spectral determination.

The remarkable different lipophilicity estimated, as the value of the logarithm of the partition coefficient of compounds 1–13 for n-octanol/water (LogP), allowed us to evaluate the possible influence of this property in the antifungal activity of each compound (Table 1).
As expected, in all compounds studied, the lipophilicity increases as the number of carbon atoms in the prenylated chain increases. Among the three natural occurring acids, the lipophilicity changes in the following order: ferulic acid $3 >$ caffeic acid $2 >$ $p$-coumaric acid $1$. The trend is also retained both in the series of geranyl esters $10 > 12 > 11$ and in the series of allyl ethers $4 > 6 > 5$. No difference in LogP value resulted in caffeic acid derivatives when the $p$-phenolic-OH or the carboxylic functionalities were protected by an allyl group (i.e., compounds $5$ and $8$) or by a 3,3'$\text{-}$dimethyl allyl chain (i.e., compounds $7$ and $13$).

### Table 1. Values of the logarithm of LogP of compounds 1–13 for n-octanol/water, estimated by ChemBioDraw Ultra 13.

| Compound | LogP | Compounds | LogP | Compounds | LogP |
|----------|------|-----------|------|-----------|------|
| 1        | 1.15 | 6         | 2.37 | 11        | 4.51 |
| 2        | 1.42 | 7         | 3.04 | 12        | 4.78 |
| 3        | 1.54 | 8         | 2.11 | 13        | 3.04 |
| 4        | 2.50 | 9         | 4.44 |           |      |
| 5        | 2.11 | 10        | 4.90 |           |      |

2.2. Antifungal Activity of the Parent Compounds 1–3 and Derivatives

The three naturally occurring hydroxycinnamic acids 1–3 and their ethers and esters derivatives 4–13 (Figure 2) were used in a preliminary in vitro screening to compare their antifungal activity against six Fusarium spp. clinical isolates associated to onychomycosis in a hospital in Milan (Italy) (Table 2).

### Table 2. List of Fusarium spp. isolates tested in this study.

| Species/Species Complex/Sequence Type (ST) | NRRL n.a | PVS-Fu n.b | Diagnosis | Isolate Source | Date |
|------------------------------------------|----------|------------|-----------|----------------|------|
| F. oxysporum/FOSC/ST33                   | 46603    | 89         | Onychomycosis | Toe            | 2004 |
| F. oxysporum/FOSC/ST33                   | 46606    | 91         | Onychomycosis | Toe            | 2005 |
| F. keratoelasticum/FSSC/ST2bb            | 46443    | 93         | Dermatomycoses | Foot          | 2004 |
| F. solani/FSSC/ST5aa                    | 44903    | 96         | Onychomycosis | Toe            | 2006 |
| F. verticillioides/FFSC                  | 46599    | 87         | Onychomycosis | Toe            | 2005 |
| F. verticillioides/FFSC                  | 46442    | 115        | Onychomycosis | Toe            | 2007 |

* NRRL n. Collection number of Agricultural Research Service (ARS); b PVS-Fu n. Collection number of Dipartimento di Agraria, Sezione Patologia Vegetale ed Entomologia, Sassari, Italy.

In the first screening, carried out on solid FMM, exposure to cinnamic acids 1–3 did not determine any significant reduction of mycelium fungal growth compared with the untreated control, with the exception of caffeic acid 2, which induced a slight inhibition on F. oxysporum 89 colony diameter (Figure S1).

In the case of FOSC and FFSC, we noted a significant inhibition of vegetative growth upon exposure to all the compounds derived from cinnamic acids 1–3 compared with the untreated control, except for compound 5, which did not induce any significant reduction of colony diameter in the two FOSC isolates. FOSC isolates were particularly sensitive (>53% and >56% of inhibition for F. oxysporum 89 and F. oxysporum 91, respectively) to compounds 4, 7, 8, 9, and 13, whereas in the case of FFSC, the most effective compounds were 5, 8, 9, 10, 11, and 13. In both species complexes, compound 13 was by far the most effective inhibitor of fungal growth, leading to complete inhibition of the two F. verticillioides isolates (Figures S1–S3).

The two representative isolates of the FSSC displayed a different level of sensitivity to compounds 4–13: compounds 4, 7, 10, 11, and 13 determined >25% inhibition of radial growth on F. keratoelasticum 93, whose vegetative growth on solid FMM was completely inhibited by compound 13, whereas F. solani 96 was only partially inhibited by compounds 10, 11 (36–30% inhibition, respectively), and 13 (63% inhibition; Figure S2).
These preliminary data demonstrate unequivocally that compound 13 has the highest antifungal activity towards all *Fusarium* strains investigated (Figures S1–S3). Citronellyl p-coumaric ester 10 was the second most effective inhibitor towards FSSC and FFSC strains, while FOSC representative isolates proved more sensitive to compound 8. Compound 9 was more effective on FOSC and FFSC representatives compared with FSSC ones. The inhibitory activity of compound 9 towards FOSC isolates was comparable to that displayed by the ethers 4 and 7, presenting a shorter prenylated chain. Overall, the ester functionality in the cinnamic acid derivatives appears more efficient in conferring inhibitory properties to the tested compounds compared with the ether one.

2.3. Determination of the Minimal Inhibitory Concentration (MIC) and Lethal Dose 50 (LD\(_{50}\)) for p-Coumaric acid 3,3′-Dimethyl Allyl Ester 13

In a further screening performed on FMM liquid medium, the minimal inhibitory concentration (MIC) and the lethal dose 50 (LD\(_{50}\)) of compound 13 and of two conventional fungicides were determined (Table 3). p-Coumaric acid 3,3′-dimethyl allyl ester 13 confirmed its remarkable good antifungal activity already shown in solid FMM compared with TRB and AmB, with an MIC range comprised between 125 and 250 µM in FOSC (Figures S4 and S5), 62 and 125 µM in FSSC (Figures S6 and S7), and 125 and 500 µM in FFSC (Figures S8 and S9) representative isolates. With respect to the LD\(_{50}\), the most effective compound was TRB (LD\(_{50}\) ranging from 2.0 to 64 µM), followed by AmB (LD\(_{50}\) ranging from 1.0 to 67.5 µM) and ester 13 (LD\(_{50}\) ranging from <7.8 to 125 µM), respectively, for almost all strains. In the case of *F. verticillioides* 115, which was less sensitive to AmB (LD\(_{50}\) 8.40–16.8 µM) than to TRB (LD\(_{50}\) 2.0–4.0 µM) and 13, the latter was able to reduce 50% of the fungal growth at a concentration <7.8 µM (Table 3, Figure S9).

| Species/Species Complex/Sequence Type (ST) | a PVS-Fu n. | d MIC (µM) | e LD\(_{50}\) (µM) | b TRB | c AmB |
|-------------------------------------------|-------------|------------|----------------|--------|--------|
| *F. oxysporum* /FOSC/(ST33)              | 89          | >125–250   | 31–62          | >256   | 8–16   |
| *F. oxysporum* /FOSC/(ST33)              | 91          | >125–250   | 62–125         | >256   | 16–64  |
| *F. keratoplasticum* /FSSC/(ST2bha)     | 93          | 62         | <7.8           | 128–256| 2.0–4.0|
| *F. solani* /FSSC/(ST35a)               | 96          | 62–125     | <7.8           | 64–128 | 2.0–4.0|
| *F. verticillioides* /FFSC               | 87          | 500        | 62–125         | >256   | >135   |
| *F. verticillioides* /FFSC               | 115         | 125–250    | <7.8           | 250    | >135   |

- a PVS-Fu n. Collection number of Dipartimento di Agraria, Sezione Patologia Vegetale ed Entomologia, Sassari, Italy; b TRB: terbinafine; c AmB: amphotericin B; d minimal inhibitory concentration (MIC) range determined visually according to mOD absorbance values (nm) detected after 48 h of incubation at 25 °C; e lethal dose 50 (LD\(_{50}\)) range determined visually according to mOD absorbance values (nm) detected after 48 h of incubation at 25 °C.

The effects of compounds 13, TRB, and AmB applied at the same concentration as the MIC determined in liquid FMM on the morphology of representative isolates of *F. solani*, *F. keratoplasticum*, *F. oxysporum*, and *F. verticillioides* were examined by optical microscopy after 72 h (Table 4). While in the untreated controls, a regular morphology of the mycelium with abundant presence of microconidia as well as normal spore germination were observed, in the presence of 250 µM of compound 13, degradation of the hyphae and vacuolisation of the cytoplasm were evident, along with an alteration of the rarely formed conidia, showing a compromised germination. The release of cell constituents was also noticeable, which can explain the progressive increase in the absorbance signal over time in liquid FMM (Figures S4–S9).

After 72 h of exposure to 256 µM TRB, a marked reduction on mycelium formation was observed, and fungal hyphae and spores showed a distorted morphology with tortuous growth (Table 4). This concentration of TRB could not totally inhibit the mycelium growth or the spore production but caused an evident swelling of conidia. The effects of TRB were
more noticeable compared with those induced by AmB at the MIC of 135 µM, causing only some mycelial distortion and fewer microconidia compared with the control (Table 4).

Table 4. Optical microscopy (100×) images of the fungal growth of Fusarium spp. after 72 h on liquid Fusarium minimal medium (FMM) amended with 250, 256, or 135 µM of p-coumaric acid 3,3′-dimethyl allyl ester 13, terbinafine (TRB), or amphotericin B (AmB), respectively, in comparison with the untreated control.

| Species          | Control 0 (µM) | Ester 13 MIC (250 µM) | TRB MIC (256 µM) | AmB MIC (135 µM) |
|------------------|----------------|-----------------------|------------------|------------------|
| *F. keratoplasticum* | ![Image](image1) | ![Image](image2) | ![Image](image3) | ![Image](image4) |
| *F. solani*      | ![Image](image5) | ![Image](image6) | ![Image](image7) | ![Image](image8) |
| *F. oxysporum*   | ![Image](image9) | ![Image](image10) | ![Image](image11) | ![Image](image12) |
| *F. verticillioides* | ![Image](image13) | ![Image](image14) | ![Image](image15) | ![Image](image16) |
3. Discussion

By introduction of a short, medium, or long prenylated chain at one of the hydroxyl functionalities of trans-cinnamic acids 1–3, a series of prenylated cinnamic derivatives 4–13 was prepared to test the hypothesis that differences in the chemical and physical proprieties would influence the antifungal activity of the compounds against clinical Fusarium spp. The presence of a conjugated double bond confers particular conformational and electronic characteristic to these compounds strongly influenced by the phenol-OH group in para position. In order to observe the effect of the hydroxylated aromatic ring on the fungicide activity, a set of cinnamic esters 10–12 was prepared by functionalisation of the corresponding carboxylic acid with citronellol, while a set of allyl cinnamic ethers 4–6 was prepared with the aim to evaluate the influence of the phenolic-OH group in para position to the alpha, beta-unsaturated carboxylic chain. No significant differences were observed in the synthesis and yields of each set of compounds with different aromatic rings, whereas lower yields in esters in comparison with ethers were achieved, evidencing the higher reactivity of the phenolic-OH group.

The Food and Drug Administration (FDA) considers trans-cinnamic acids 1–3 as “generally recognized as safe” (GRAS), enabling their use in the field of food additives [40]. Compounds 1–3 are commercially available at an affordable price and can be obtained by direct extraction from plants biomass where the compounds are the main components [41] or by chemical and biotechnological processes [42]. Besides cinnamic acids 1–3, prenylated cinnamic ester 13 and ethers 7 and 9 are plant components, whose extracts were studied for their remarkable biological properties [43]. In particular, compounds 9 and 13 are present in propolis, a source of valuable compounds with antioxidant and antimicrobial activity [44]. Compound 9 is not toxic to human cells and presents antitumoral and anti-inflammatory activities, in addition to acting as an inhibitor of biofilm formation by oral pathogenic bacteria [45]. While 4’-geranyloxy ferulic acid 9 is generally extracted from citrus fruit, quinoa seeds, and several vegetable oils, compound 13 was only detected in propolis extract. Propolis, produced by honeybees, is a very complex mixture composed of 50% resin, 30% wax, 10% essential oil, and 5% of polyphenols as flavonoids, terpenes, fatty acids, stilbenes, β-steroids, cinnamic acids, and their prenylated derivatives [46,47]. Change in chemical composition of propolis is frequently observed [44]. A few studies have been conducted on the antifungal and antibiofilm activity of propolis against onychomycosis caused by Fusarium spp. [48,49], but no studies aimed to identify the active component of the propolis extract against these fungi.

In a previous article reporting the activity of natural phenols against clinical Fusarium spp., we observed that the percentage of growth inhibition measured in liquid medium (Vogel’s) and solid (PDA) was comparable [50]. To achieve full solubilisation of compounds 1–13 at 0.5 mM, the preliminary screening was carried out with a sustainable solid medium based on gellan/water, where each compound was solubilized in a 0.1% water/gellan solution. In the preliminary in vitro screening of compounds 1–13 against Fusarium spp., cinnamic acids 1–3 were generally ineffective, whereas significant growth inhibition was achieved by prenylated derivatives 4–13, evidencing ester 13 as the most active. Among the Fusarium spp. investigated, F. solani was the most active against compounds 1–13, whereas F. verticillioides was the most sensitive, in accordance with data present in the literature for these species. In fact, Fusarium spp. are increasingly reported as resistant to many antifungal compounds in vitro; among them, F. solani is considered as the most resistant taxon, albeit some reports pointed out that the resistance may be species- and even isolate-dependent [51].

The antifungal activity of compounds 1–13 may be explained by the key role played by some moieties of their structure. The prenylated chain present in compounds 4–13 has the ability to penetrate and to accumulate within the fungal cell membrane, resulting, according to the size, in the disruption of its integrity as generally acknowledged for prenylated phenols [36–38]. The position and size of the prenylated chain in the studied compounds appear crucial for their antifungal activity. Although we did not perform a
proper bioavailability assay, we observed a detrimental effect on the fungal membrane when treated with compound 13 (Table 4).

Esters were more active than ethers as inhibitors of all tested Fusarium spp. Ester 13 was definitely more active than the corresponding ether 7 containing the same 3,3′-dimethyl allyl moiety, even though an identical lipophilicity was measured for both compounds (LogP 3.04). In the esters series, the alcoholic unit represented by a 3,3′-dimethyl allyl chain (compound 13) was more active than the citronellyl one (compound 10), a substituent that significantly increases the lipophilicity of the molecule (LogP 4.9).

We suppose that different prenylated chains may change the bioavailability of the compound influencing the hydrolytic degradation of the prenylated esters within the fungal cell. In fact, hydrolytic degradation, mediated by fungal enzymes, of the esters in the parent cinnamic acid and the corresponding alcohol cannot be ruled out. In esters 11 and 10, a too long prenylated chain could be partially metabolized by the fungus at the first stages of contact, whereas in ester 13, the hydrolysis would take a longer time, allowing it to reach sensitive compartments of the fungal cell where the prenyl alcohol may exert its antifungal activity. A similar effect has been reported by farnesol on F. keratoplasticum, which is associated with biofilm formation in hospital water systems and internal pipelines: this prenylated alcohol has a remarkable anti-biofilm activity; causes the destruction of hyphae and of the extracellular matrix; and prevents the adhesion of conidia, filamentation, and the formation of biofilm [52].

Compounds 4–13 contain an α,β-unsaturated Michael acceptor pharmacophore effective in interacting with nucleophiles present in the fungal cell; nevertheless, this feature is not exhaustive for the antifungal activity. The presence of a free phenol-OH in para position would play a key role in the radical scavenging and stabilisation of the radical by electronic delocalisation along the structure. In general, we observed that compounds with a catechol and guaiacyl ring favouring an intramolecular hydrogen bond and hampering the availability of the H donor to scavenge radicals were less active as antimycotic (compounds 5, 8, and 12).

The antifungal activity of compound 13 was compared with that of TRB and AmB, two of the most effective conventional fungicides for clinical use [53]. TRB and AmB were applied at clinical dosage ranging between 2–256 µM and 1–135 µM, respectively, whereas compounds 13 was amended at concentrations comprised between 7.8 and 500 µM. Both TRB and AmB interact at the level of fungal cell membrane, the first one by inhibiting squalene epoxidase, a key step along the ergosterol biosynthesis pathway, and the second one by a complex interaction with phospholipid bilayers [54]. Our results demonstrate that compound 13 presents MIC and LD50 values against F. verticillioides 115 and F. oxysporum 89 that are consistent with those reported for AmB. Similarly, while AmB was indeed the most effective compound in terms of MIC and LD50 against F. keratoplasticum 93 and F. solani 96, the antifungal efficacy of compound 13 against these members of the FSSC was comparable to that of terbinafine. Besides its remarkable biological activity, ester 13 presents some attractive advantages; that is, it is as natural compound with a simple structure, a straightforward synthesis, low production cost with easy recovery of the starting materials. Considering the increasing frequency of multi-drug resistance patterns in opportunistic Fusarium spp. [55], the development of compound 13 as an effective antifungal drug represents a valuable alternative to the conventional therapeutic agents in onychomycosis treatment.

The results of this study provide useful insights to the optimal design of the structure of cinnamic esters with improved antifungal properties. Although cinnamic acids and their derivatives have been studied on some plant pathogenic fungi [56], to the best of our knowledge, no investigation was conducted on prenylated cinnamic esters and ethers on clinical Fusarium spp., thereby offering an intriguing opportunity in drug repositioning strategy.
4. Materials and Methods

4.1. Chemical Synthesis

4.1.1. General

Unless otherwise noted, starting materials and reagents were obtained from commercial suppliers and were used without further purification. Melting points were determined on a Büchi 530 apparatus and are uncorrected. All $^1$H-NMR and $^{13}$C-NMR spectra were recorded in CDCl$_3$ (if not otherwise indicated) solution at 399.94 MHz and 75.42 MHz, respectively, with a Varian VXR 5000 spectrometer (Varian, Palo Alto, CA USA). Chemical shifts are given in ppm ($\delta$); multiplicities are indicated by s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), or dd (doublet of doublets). Elemental analyses were performed using an elemental analyser model 240 C (Perkin-Elmer, Waltham, MA USA). Acetone was freshly distilled from CaCl$_2$. Flash chromatography was carried out with silica gel 60 (230–400 mesh; VWR; Radnor, AF, USA) eluting with appropriate solution in the stated v:v proportions. Analytical thin-layer chromatography (TLC) was performed with 0.25 mm thick silica gel plates (Sigma Aldrich, Munich, Germany). All reactions were monitored by TLC performed on 0.2 mm thick silica gel plates (60 F254 Merck). Microwave reactions were carried out on a MW instrument (CEM-Discover SP MW, Matthews, NC, USA). Melting points were determined on a 530 apparatus (Büchi, Flawil, Switzerland) and are uncorrected. The purity of all new compounds was judged to be >98% by $^1$H-NMR spectral determination.

Lipase from Candida antarctica (Novozym 435 CAL-B) is immobilized on a macroporous polyacrylic resin beads (recombinant, expressed in Aspergillus niger, activity $\geq$ 5000 PLU/g (propyl laurate units/g) and purchased from Merck (Milan, Italy). Compound 8 was prepared according to the literature [57]. Lipophilicity of the compounds was estimated using the logarithm of the partition coefficient for n-octanol/water (log P), which was calculated using 403 ChemBioDraw Ultra 13.0.

4.1.2. General Procedure for the Synthesis of Compounds 10–13

Ethyl chloroformate (2 eq for 10, 12, and 13 or 3 eq for 11) and triethylamine (2 eq for 10, 12, and 13 or 3 eq for 11) were added to a suspension of appropriate cinnamic acid (1 eq) in dichloromethane (10 mL) and stirred for 1 h at $-30^\circ$C until all of the starting material disappeared, as determined by TLC. Appropriate alcohol (1 eq) and 4-dimethylaminopyridine (0.2 eq) were then added, and the mixture was stirred at room temperature for 6 h. The reaction mixture was acidified with hydrochloridric acid (10% solution) and extracted with dichloromethane (3 $\times$ 50 mL), and the organic phases were combined and dried over anhydrous sodium sulphate. The crude product was concentrated under reduced pressure and purified by flash chromatography using a 1:1 mixture of petroleum ether/ethyl acetate as eluent to give the pure ester.

$^{(E)}$-3,7-Dimethyloct-6-en-1-yl 3-(4-hydroxyphenyl)acrylate 10: oil; 44%; $[\alpha]_D^{20}$ 0.5 (c = 0.9, CHCl$_3$); $^1$H-NMR $\delta$ 0.94 (d, $J$ = 6.4 Hz, 3H), 1.22 (m, 1H), 1.38 (m, 1H), 1.51 (m, 1H), 1.60 (m, 1H), 1.61 (s, 3H), 1.67 (s, 3H), 1.76 (m, 1H), 1.99 (m, 2H), 4.26 (m, 2H), 5.11 (m, 1H), 6.28 (d, $J$ = 16.0 Hz, 1H), 6.81 (m, Ar, 2H), 7.42 (m, Ar, 2H), 7.63 (d, $J$ = 16.0 Hz, 1H); $^{13}$C-NMR $\delta$ 17.66, 19.43, 25.40, 25.73, 29.54, 35.48, 36.99, 63.40, 114.97, 115.99, 124.57, 126.63, 130.07, 131.37, 145.12, 158.51, 168.44; Anal. Calcd. for C$_{19}$H$_{26}$O$_3$: C, 75.46; H, 8.67; Found: C, 75.44; H, 8.60.

$^{(E)}$-3,7-Dimethyloct-6-en-1-yl 3-(3,4-dihydrophenyl)acrylate 11: brown solid; 47%; mp 100–101 $^\circ$C; $[\alpha]_D^{20}$ 2.9 (c = 0.4, CHCl$_3$); $^1$H-NMR $\delta$ 0.93 (d, $J$ = 6.4 Hz, 3H), 1.21 (m, 1H), 1.36 (m, 1H), 1.52 (m, 1H), 1.59 (m, 1H), 1.60 (s, 3H), 1.67 (s, 3H), 1.74 (m, 1H), 1.99 (m, 2H),
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4.30 (m, 2H), 5.09 (m, 1H), 6.28 (d, J = 15.6 Hz, 1H), 6.88 (d, J = 8.4 Hz, Ar, 1H), 6.99 (dd, J = 2.1, 8.4 Hz, Ar, 1H), 7.12 (d, J = 2.1 Hz, Ar, 1H), 7.59 (d, J = 15.6 Hz, 1H); 13C-NMR δ 17.66, 19.42, 25.37, 25.72, 29.51, 35.42, 36.97, 63.61, 114.44, 115.14, 115.46, 122.46, 124.52, 127.17, 131.42, 144.03, 145.56, 146.78, 168.69; Anal. Calcd. for C14H12O4: C, 71.67; H, 8.23; Found: C, 71.60; H, 8.26.

(E)-3,7-Dimethyloct-6-endo-1-yl 3-(4-hydroxy-3-methoxyphenyl)acrylate 12: oil; 37%; [α]D20 0.6 (c = 0.4, CHCl3); 1H-NMR δ 3.79 (s, 3H), 5.37 (bs, 1H), 6.28 (d, J = 16.0 Hz, 1H), 6.85 (m, Ar, 2H), 7.42 (m, Ar, 2H), 7.63 (d, J = 16.0, 1H), 13C-NMR δ 51.75, 114.52, 115.91, 125.98, 129.89, 144.79, 158.12, 167.65. Anal. Calcd. for C14H12O3: C, 67.41; H, 5.66; Found: C, 67.53; H, 5.56.

(E)-Methyl 3-(3,4-dihydroxyphenyl)acrylate 15: brown solid; 88%; mp 155–156 ºC [59] 160 ºC; 1H-NMR δ 3.80 (s, 3H), 6.25 (d, J = 16.0 Hz, 1H), 6.87 (d, J = 8.4 Hz, Ar, 1H), 7.01 (dd, J = 2.0, 8.4 Hz, Ar, 1H), 7.07 (d, J = 2.0 Hz, Ar, 1H), 7.58 (d, J = 16.0 Hz, 1H); 13C-NMR δ 51.78, 114.33, 115.27, 115.49, 122.46, 127.52, 143.74, 145.03, 146.28, 168.18. Anal. Calcd. for C14H12O3: C, 61.85; H, 5.19; Found: C, 62.05; H, 5.78.

(E)-Methyl 3-(4-hydroxy-3-methoxyphenyl)acrylate 16: brown solid; 92%; mp 62–64 ºC [60] 65 ºC; 1H-NMR δ 3.75 (s, 3H), 3.83 (s, 3H), 6.14 (bs, 1H), 6.25 (d, J = 16.0 Hz, 1H), 6.86 (d, J = 8.0 Hz, Ar, 1H), 6.96 (d, J = 2.0 Hz, Ar, 1H), 6.96 (dd, J = 2.0, 8.0 Hz, Ar, 1H), 7.58 (d, J = 16.0 Hz, 1H); 13C-NMR δ 51.64, 55.84, 109.52, 114.72, 114.81, 122.98, 126.78, 145.12, 146.92, 148.12, 167.92. Anal. Calcd. for C14H12O4: C, 63.45; H, 5.81; Found: C, 63.51; H, 5.76.

(E)-Ethyl 3-(4-hydroxy-3-methoxyphenyl)acrylate 17: brown solid; 87%; mp 70–72 ºC [61] 73–74 ºC; 1H-NMR δ 1.32 (t, J = 7.1 Hz, 3H), 4.26 (q, J = 7.1 Hz, 2H), 6.39 (d, J = 16.0 Hz, 1H), 7.35 (m, Ar, 2H), 7.47 (m, Ar, 2H), 7.58 (d, J = 16.0 Hz, 1H); 13C-NMR δ 14.43, 60.74, 119.14, 124.58, 129.54, 132.22, 133.52, 143.29, 166.78. Anal. Calcd. for C15H12O3: C, 68.74; H, 6.29; Found: C, 68.81; H, 6.34.

4.1.4. Procedure for the Synthesis of Compound 13 with Lipase

To a solution of 17 (1 eq) in cyclohexane (2.5 mL), 3,3-dimethylallyl alcohol (2 eq) was added. The reaction mixture was stirred at 60 ºC for 15 min at a speed of 300 rpm. The reaction was initiated by adding a known fixed quantity of lipase (100 mg). The progress of the reaction was monitored by TLC using a 1:1 mixture of petroleum ether/ethyl acetate as eluent. After three days, the starting material was still present and another aliquot of
lipase (100 mg) was added, and the mixture was left stirring at 60 °C for two additional days. The reaction mixture was filtered over Buchner funnel, solvent concentrated under reduced pressure, and purified by flash chromatography using a 1:1 mixture of petroleum ether/ethyl acetate as eluent to obtain compound 13 (0.19 g, 80% yield).

4.1.5. General Procedure for the Synthesis of Compounds 4, 6, 7, and 9

Compound 14 or 15 or 16 (1 eq) was dissolved in dry acetone (15 mL) and then anhydrous potassium carbonate (1 eq) and appropriated alkenyl bromide (1 eq) were added. The resulting mixture was stirred at 50 °C for 12 h, then sodium hydroxide 2 N (15 mL) was added and the reaction mixture was stirred at 90 °C for an additional 3 h. The cooled solution was acidified to pH 2 with hydrochloridric acid (10% solution) and extracted with dichloromethane (3 × 50 mL). The collected organic phases were dried over anhydrous sodium sulphate and evaporated to dryness to give the pure ether.

(E)-3-(4-(Allyloxy)phenyl)acrylic acid 4: white solid; 85%; mp 161–162 °C (62) 160 °C; 1H-NMR δ 4.56 (d, J = 5.2 Hz, 2H), 5.29–5.44 (series of m, 2H), 6.03 (m, 1H), 6.29 (d, J = 16.0 Hz, 1H), 6.91 (m, Ar, 2H), 7.49 (m, Ar, 2H), 7.73 (d, J = 16 Hz, 1H); 13C-NMR δ 68.85, 114.72, 115.13, 118.08, 126.91, 130.07, 132.66, 146.67, 160.73, 172.55. Anal. Calcd. for C12H12O3 C, 72.70; H, 7.93; Found: C, 72.80; H, 7.92.

(E)-3-(4-(3-Methoxyphenyl)acrylic acid 5: white solid; 70%; mp 131–132 °C (63) 131–133 °C; 1H-NMR δ 3.91 (s, 3H), 4.66 (m, 2H), 5.30–5.44 (series of m, 2H), 6.08 (m, 1H), 6.29 (d, J = 16.0 Hz, 1H); 6.86 (d, J = 8.4 Hz, Ar, 1H), 7.08 (dd, J = 2.0, 8.4 Hz, Ar, 1H), 7.09 (d, J = 2.0 Hz, Ar, 1H), 7.71 (d, J = 16.0 Hz, 1H); 13C-NMR δ 55.92, 69.70, 110.36, 112.71, 114.90, 118.51, 122.92, 127.14, 132.61, 147.01, 149.52, 150.46, 172.72. Anal. Calcd. for C13H14O4 C, 66.66; H, 6.02; Found: C, 66.87; H, 6.12.

(E)-3-(4-((3-Methylbut-2-en-1-yl)oxy)phenyl)acrylic acid 6: white solid; 69%; mp 146–147 °C (16) 148–150 °C; 1H-NMR δ 1.73 (s, 3H), 1.79 (s, 3H), 4.52 (d, J = 6.4 Hz, 2H), 5.47 (s, 1H), 6.28 (d, J = 16 Hz, 1H), 6.89 (d, J = 8.8 Hz, Ar, 2H), 7.47 (d, J = 8.4 Hz, Ar, 2H), 7.73 (d, J = 16 Hz, 1H); 13C-NMR δ 25.81, 29.19, 64.91, 114.62, 115.04, 119.11, 126.67, 130.43, 138.73, 146.60, 161.04, 171.36. Anal. Calcd. for C14H16O3 C, 72.39; H, 6.94; Found C, 72.59; H, 6.03.

(E)-3-(4-(((E)-3,7-Dimethylocta-2,6-dien-1-yl)oxy)-3-methoxyphenyl)acrylic acid 7: white solid; 85%; mp 59–60 °C (64) 60–61 °C; 1H-NMR δ 1.63 (s, 3H), 1.70 (s, 3H), 1.77 (s, 3H), 2.01–2.24 (series of m, 4H), 3.95 (s, 3H), 4.62 (m, 2H), 5.11 (m, 1H), 5.44 (m, 1H), 6.33 (d, J = 16.0 Hz, 1H), 6.86 (d, J = 7.6 Hz, Ar, 1H), 7.01–7.15 (series of m, Ar, 2H), 7.37 (d, J = 16.0 Hz, 1H); 13C-NMR δ 16.70, 17.70, 25.70, 26.20, 39.52, 55.91, 65.82, 109.91, 112.52, 114.71, 119.11, 123.01, 123.71, 126.81, 131.44, 141.22, 147.01, 149.52, 150.83, 172.11. Anal. Calcd. for C20H20O4 C, 72.70; H, 7.93; Found: C, 72.80; H, 7.92.

4.1.6. Synthesis of Compound 5

(E)-3-(4-(Allyloxy)-3-hydroxyphenyl)acrylic acid 5: Compound 15 (0.5 g, 2.57 mmol) was dissolved in dry acetone (15 mL) and then anhydrous potassium carbonate (0.35 g, 2.57 mmol) and allyl bromide (0.31 g, 2.57 mmol) were added. The resulting mixture was stirred at 50 °C for 12 h. The cooled solution was acidified to pH 2 with hydrochloridric acid (10% solution) and extracted with dichloromethane (3 × 50 mL). The collected organic phases were dried over anhydrous sodium sulphate and evaporated to dryness to give compound 18 as a white solid (0.47 g, 78%).

(E)-Methyl 3-(4-(allyloxy)-3-hydroxyphenyl)acrylate 18: mp 95–96 °C (65) 94–95 °C; 1H-NMR δ 3.78 (s, 3H), 4.65 (d, J = 5.6 Hz, 2H), 5.33–5.43 (series of m, 2H), 5.69 (s, 1H), 6.08 m, 1H), 6.30 (d, J = 15.6 Hz, 1H), 6.83 (d, J = 8.4 Hz, Ar, 1H), 6.99 (dd, J = 2.0 8.4 Hz, Ar, 1H), 7.14 (d, J = 2.0 Hz, Ar, 1H), 7.61 (d, J = 15.6 Hz, 1H); 13C-NMR δ 51.59, 69.80, 11.84, 113.21, 115.94, 118.80, 121.64, 123.13, 128.20, 132.26, 144.60, 146.02, 147.39, 167.68. Anal. Calcd. for C14H15O4 C, 67.73; H, 6.50; Found: C, 67.52; H, 6.42. To compound 18 (0.47 g, 2.00 mmol) in a 3:1 solution of MeOH:H2O, sodium hydroxide 2 N (15 mL) was added and the mixture was stirred at 90 °C for 3 h. The cooled solution was acidified to pH 2 with
hydrochloridric acid (10% solution) and extracted with dichloromethane (3 × 50 mL). The collected organic phases were dried over anhydrous sodium sulphate and evaporated to dryness to give the pure 5 as white solid (0.33 g, 75%); mp 184–185 °C; 1H-NMR δ (acetone d6) 4.66 (m, 2H), 5.26 (m, 1H), 5.46 (m, 1H), 6.07 (m, 1H), 6.32 (d, J = 16 Hz, 1H), 7.01 (d, J = 8.4 Hz, Ar, 1H), 7.09 (dd, J = 2.4, 8.4 Hz, Ar, 1H), 7.19 (d, J = 2.4, Hz, Ar, 1H), 7.56 (d, J = 16 Hz, 1H); 13C-NMR δ 64.41, 112.87, 113.95, 115.94, 117.05, 121.08, 128.03, 133.44, 144.61, 147.08, 148.52, 167.09. Anal. Calcd. for C12H12O4 C, 65.45; H, 5.49; Found: C, 65.52; H, 5.32.

4.2. Fungal Strains and Culture

Two monosporic isolates collected from human samples from an Italian hospital (Table 2) were selected as representative of each Fusarium oxysporum, F. solani, and F. fujikuroi species complexes. Conidial suspensions of each strain were pre-cultured in a carboxymethyl cellulose medium (CMC; [66]) for 5 days on a rotary shaker at 24 °C and 180 rpm. Cultures were filtered through four layers of sterile cheesecloth, and spores were collected by centrifugation, adjusted to 1 × 10⁶ colony-forming units (CFU)/mL in sterile water, and used as inoculum.

4.3. Evaluation of the Antifungal Activity of Compounds 1–13 in FMM Solid Medium

A total of 13 phenolic compounds (Table 1) were tested for their antifungal activity against the six Fusarium spp. isolates (Table 2) in Fusarium minimal medium (FMM) [67]. Each phenolic compound was resuspended in H2O/gellan 0.1 % solution and sonicated at room temperature for 1 h at 80 Hz (Elmasonic P 180 H, Elma Schmidbauer GmbH, Germany). Solid FMM with nitrate sodium NaNO3 as nitrogen source was distributed into 90 mm Petri dishes (15 mL/Petri dish) and amended with each compound at a final concentration of 0.5 mM at a temperature of 45 °C. Ten microliters of the conidial suspension of each strain were spotted onto the center of the Petri dish amended FMM. Antifungal activity of each compound was measured after 5 d of growth at 25 °C in the dark and expressed as the colony diameter (percentage relative to control). Three replicates were prepared for each isolate/inhibitor combination and the experiment was repeated once.

4.4. Evaluation of the Antifungal Activity of p-Coumaric Acid 3,3′-Dimethyl Allyl Ester (13) in FMM Liquid Medium

The conventional antifungal drugs used in the study were AmB and TRB. AmB was purchased by Sigma Aldrich (A2942, Germany) as a standard solution. TRB was extracted from Terbinafine Hexal 250 mg tablets by fine crushing and dissolution in a solution 1:2 (v/v) dichloromethane and water. The emulsion was stirred at room temperature until two phases clearly appeared. The organic phase was extracted and dried on Na2SO4 and the TRB was recovered in neat form after evaporation of the solvent under vacuum. NMR spectra of the solid extract confirmed the presence of TRB with a purity ∼98%. TRB was dissolved in 60% ethanol/H2O (v/v), while AmB was diluted with water to reach the desired concentration and was frozen in aliquots at −20 °C. AmB and TRB concentrations were selected according to clinical dosage and standard experimental procedures with some modifications [68].

The minimal inhibitory concentration (MIC) and the lethal dose 50 (LD50) of each strain were assayed by a standardized micro-dilution method in the 96-well plate. Two-fold serial dilutions of each antifungal agent in liquid FMM in a total volume of 200 µL were tested. Further, 10 µL of fungal spore suspension (4 × 10⁶ CFU/mL) was added. A blank control with water was used for each treatment. The optical density mOD of each microplate well was measured at 2 h intervals during 72 h of incubation with a microplate spectrophotometer SpectrostarNano (Euroclone, Germany) at a 595 nm wavelength. The inhibitory activity of each compound was expressed as MIC, representing the lowest concentration of active ingredient (µM) that is sufficient to inhibit the absorbance signal, whereas the LD50 of each compound was calculated as the concentration of active ingredient...
The experiments were repeated at least two times in quadruplicate.

4.5. Optical Microscopy Examination

A drop (15 µL) of the total volume present on the wells corresponding to the MIC of each isolate/compound combination was pipetted after 72 h of incubation onto a glass slide. A clean glass cover slip was placed on the sample prepared with emulsion oil. Each slide was examined at 100× for the presence of mycelium, branched hyphae, fialides, microconidia, and germinating spores, using an optical microscope (LEICA ICC50) at a scale of 20 µm.

4.6. Data Acquisition and Analysis

In the first screening, an analysis of variance (one-way ANOVA) followed by multiple comparisons by Tukey HSD test at the significance level $p < 0.05$ using Minitab for Windows, release 17 was performed.

In the second screening, data were recorded and analysed with Mars Data Analysis Software, BMG Labtech, and exported to Microsoft Excel for generation of the graphs. Graphs for the determination of the MIC and LD$_{50}$ (Figures S4–S9) were generated for an incubation time between 0 h and 48 h because, after this interval time, the drug free-test (control) curve started to reach the stationary phase for almost all strains investigated. Optical microscopy images were captured and treated with LAS V4.13 Leica application software.

5. Conclusions

The design of cinnamic derivatives 4–13 was focused on both electronic and steric modification of the parent compounds 1–3 by esterification and etherification reaction with bioactive prenylated chains, with the aim to enhance the antifungal activity of the final compound. Compounds 1–3 are commercially available at a reasonable price and offer a successful example of repositioning of natural compounds. In this study, we provided data that may contribute to increasing the knowledge about the promotion of the importance of antifungal susceptibility testing. $p$-Coumaric acid 3,3′-dimethyl allyl ester 13, a component of propolis, showed good antifungal activities against *Fusarium* spp., causing onychomycosis, and identifies prenylated hydroxy cinnamic acids as interesting pharmacophore for developing new drugs effective against this pathology. The activity of this compound will be investigated over a larger number of isolates belonging to different species complexes and haplotypes. Furthermore, the mechanism of action of compound 13 needs to be fully characterized and possibly tested in combination with other bioactive molecules that may be enabled to reach their target within the fungal cell.

Noteworthy, this study cannot be adopted as a clinical guideline, and the MIC values obtained must be tested in an appropriately designed clinical study.

Supplementary Materials: The following are available online. Figures S1–S3: Antifungal activity of compounds 1–13 against FOSC, FFSC, and FFSC, respectively. Figures S4–S9: MIC and LD$_{50}$ ranges expressed as absorbance (milliOD) at 595 nm at 48 h of ester 13 (A), TRB (B), and AmB (C) against FOSC, FFSC, and FFSC strains, respectively.

Author Contributions: Conceptualization, G.D. and Q.M.; methodology, S.O., V.B., M.A.D., and D.F.; software, S.O.; validation, S.O., P.C., and D.F.; formal analysis, S.O., V.B., S.C., W.C., P.C., and D.F.; resources, Q.M. and D.F.; data curation, S.O., S.C., and P.C.; writing—original draft preparation, G.D., M.A.D., S.O., and S.C.; writing—review and editing, G.D. and Q.M.; funding acquisition, Q.M. and D.F. All authors have read and agreed to the published version of the manuscript.

Funding: This study has been supported by the following projects: CNR-CONICET Bilateral project 2016–2018; RASSR73282: “IDEASS: Approcci innovativi nella difesa delle colture agrarie: studi in silico, selezione e sintesi di composti a ridotta tossicità per il contenimento di funghi”; CUP:
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