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Synthesis and antifungal activity of carvacrol and thymol esters with heteroaromatic carboxylic acids

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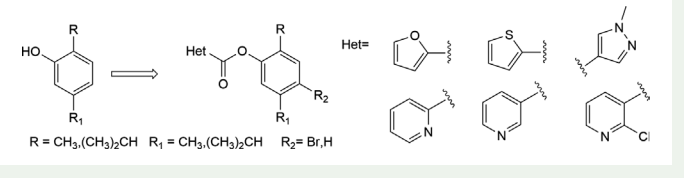
ABSTRACT

Aiming to obtain the more effective pathogen inhibitive ingredients and explore the influence of introducing different heterocyclic units to carvacrol and thymol esters, twenty ester derivatives with different heterocyclic units were synthesized. And the *in vitro* antifungal activity of title compounds against five plant pathogenic fungi was evaluated by mycelium growth rate method. The results showed that some carvacrol and thymol esters showed good to excellent antifungal activity, and compound 9d (4-bromo-5-isopropyl-2-methylphenyl picolinate) exhibited a broad antifungal spectrum. Preliminary study indicated that the introduction of furan, thiophene and pyridine unit could enhance the antifungal activity of carvacrol and thymol esters against *Botrytis cinerea* and a bromine atom on the *para* position of benzene moiety could enhance their antifungal activity.



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Carvacrol; thymol; ester derivatives; heterocyclic compound; antifungal activity

1. Introduction

Plant disease cause major losses in agriculture, which are primarily controlled by application of synthetic fungicides. But the overuse of synthetic pesticides can lead to fungicides resistance, food and environmental pollution (Aliferis and Jabaji 2011), which encourages the scientists and researchers to develop novel and improved compounds (Ehler 2006). Natural products are attracting more attention in research and development of pesticides because of their different structures, various bioactivities, less side effects, biodegradable properties and rich source (Dayan et al. 2009). Now, a considerable amount of natural resources have been applicated in plant disease control (Copping and Duke 2007). Generally, natural

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compounds are not commercially available for non-drug-like physicochemical properties such as volatility, stability and water solubility (Dayan et al. 2009). An alternate strategy to combat this problem is combining two or more single active agents or compounds in one molecule. Hybrid molecules with dual mode of action have been used for generating new and more active compounds and drugs (Borate et al. 2011; Pete et al. 2012; Shaveta and Singh 2016).

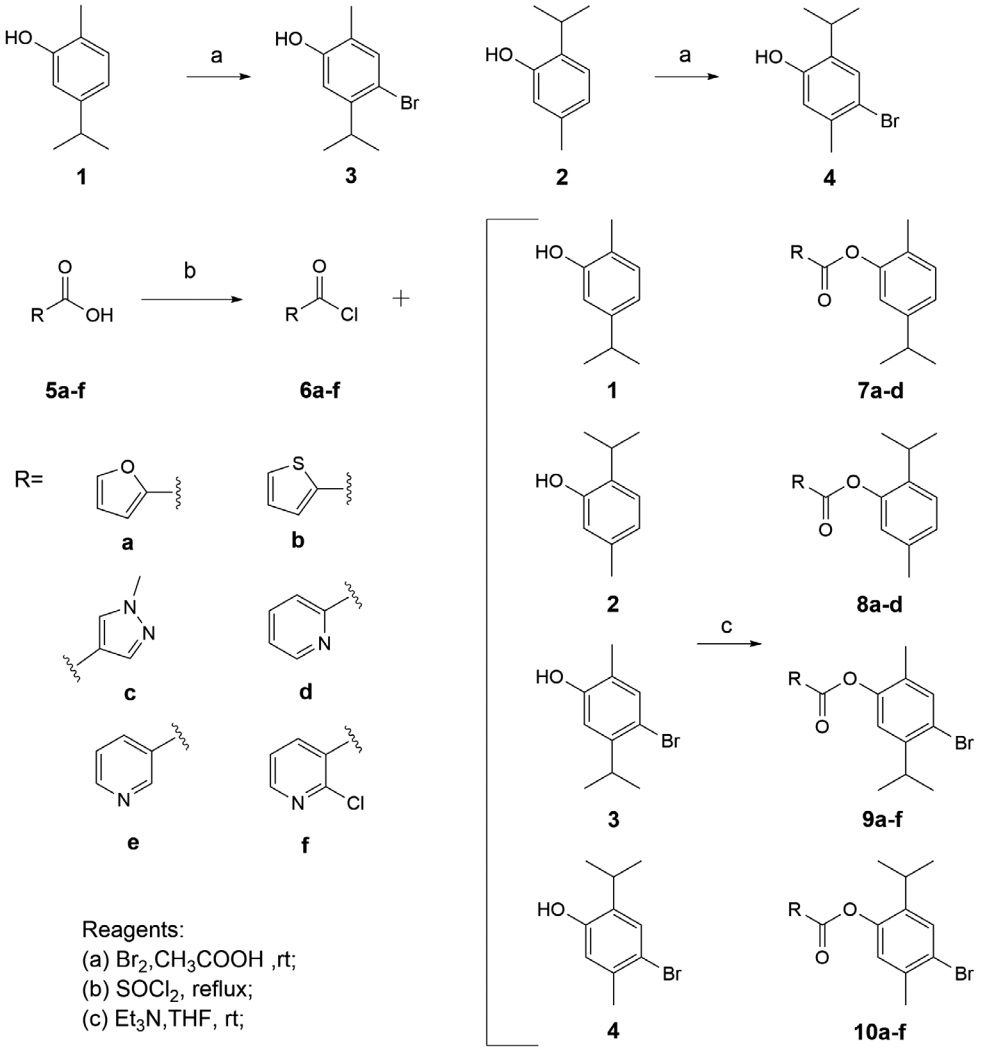
Carvacrol and thymol are constituent of essential oils produced by numerous aromatic plants such as the genus *Origanum* (Stefanakis et al. 2013), *Thymus* (Porte and Godoy 2008), *Lippia* (Bueno-Durán et al. 2014) and *Satureja* (Yousefzadi et al. 2012; Wesolowska et al. 2015). The two natural smaller molecules have exhibited potential biological activity, such as antibacterial and antifungal activity (Taha and Azeiz 2010; Friedman 2014; Suntres et al. 2015), and can potentially be used as antifungal agents against plant pathogenic fungi (Vázquez et al. 2001; Soković et al. 2002; Numpaque et al. 2011). And in our previous studies, carvacrol and thymol had showed a significant inhibitory effect on the mycelial growth of several plant pathogenic fungi, and their ester derivatives were more effective. Thus, we suggested more ester derivatives of them should be synthesized to obtain the more effective compounds (Wang et al. 2018). Heterocyclic compounds had played an important role in molecular design of pesticides due to their diversity of chemical structure, broad-spectrum of biological activity, low toxicity and high activity (Liu et al. 2007). Therefore, it attracted our interests to design carvacrol and thymol esters with heterocyclic units such as furan, thiophene, methylpyrazole and pyridine. Meanwhile, it was reported that the antifungal activity of halogenated thymol derivatives is different from thymol (Kaur et al. 2013). Considering the electronic and space effects of halogen atom may greatly affect the conjugation system and coplanarity of the compounds, brominated carvacrol and thymol esters were also synthesized.

In this study, twenty carvacrol and thymol esters were synthesized by linking the carvacrol or thymol hydroxyl moiety to the carboxyl moiety of heteroaromatic carboxylic acids. And their antifungal activity against five kinds of important plant pathogenic fungi (*Alternaria solani*, *Botrytis cinerea*, *Fusariu oxysporum*, *Pyricularia oryzae*, and *Rhizoctonia solani*) were also evaluated.

2. Results and discussion

2.1. Synthesis and characterization

The synthetic routes to carvacrol and thymol ester derivatives are outlined in Scheme 1. Bromo-carvacrol and bromo-thymol (compounds **3**, **4**) were respectively prepared via bromination of **1** and **2**, according to the procedure described by Soderberg (Soderberg and Fields 1996). Compounds **6a-f** were prepared by the reaction of heterocyclic carboxylic acids with $SOCl_2$, according to the reported procedure (Cui et al. 2014). Furthermore, carvacrol, thymol and their brominated derivatives were respectively reacted with compounds 6a-f to afforded ester derivatives (compounds **7a-d**, **8a-d**, **9a-f**, **10a-f**) in a yield of 53–86%. The chemical structure of compound **3**, **4** and all the ester derivatives were elucidated on the basis of ^{1}H NMR, ^{13}C NMR, and HR-ESI-MS analyses.



Scheme 1. Synthetic route to carvacrol and thymol esters with heteroaromatic carboxylic acids.

2.2. Antifungal assay

The *in vitro* antifungal activity of all the synthetic compounds against five plant pathogenic fungi was evaluated by mycelium growth rate method at 10 μg·mL^{-1} and 50 μg·mL^{-1}, and the results are listed in Table S1 in the supplementary materials. Preliminary antifungal assay indicated that all the synthetic compounds exhibited antifungal activity to a certain extent and significant antifungal activity at the higher concentration (50 μg·mL^{-1}). At first, all synthetic compounds exhibited low antifungal activity against *A. Solani* and *F. oxysporum*, and their inhibition ratios were below 80 percent at test concentrations. Then, some compounds exhibited higher antifungal activity than chlorothalonil, such as compounds **8a**, **9d**, **10a**, **10**b and **10d** against *B. Cinerea*, and compounds **7a**, **7b** and **9d** against *R. Solani*. Carvacrol and thymol exhibited low active against *P. oryzae*, but their esters compounds **9d**, **9e** and **10b** exhibited higher antifungal activity than them. Interestingly, compound **10b** exhibited

100% inhibition ratio against *P. oryzae* at 50 μg·mL^{-1}, but it exhibited low inhibition ratio (5.48 ± 0.27) at 10 μg·mL^{-1}.

The antifungal activity of the synthetic compounds against *B. cinerea* were related to their chemical structure. At first, esters with furan, thiophene and pyridine unit exhibited high potential antifungal activity, and esters with methylpyrazole unit exhibited low antifungal activity. Moreover, most of the thymol esters exhibited higher antifungal activity than carvacrol esters did. For example, compound **10b**, a thymol ester with thiophene unit, was more effective than its isomer compound **9b**. The exactly similar results were also observed in esters with furan, methylpyrazole or pyridine unit. On the other hand, brominated esters showed higher inhibition ratios. For example, compound **8b**, a thymol ester without bromine substituent, was less effective than its brominated compound **10b**. However, the chlorine atom in pyridine unit of synthetic compounds could not enhance their antifungal activity. The antifungal activity of compounds **9e** and **10e** were not higher than compounds **9d** and **10d**. Besides, the antifungal activity of synthetic compounds was affected by the position of ester bond at the heterocyclic moiety. The compounds connected with ester bond at *ortho* position in pyridine unit (compounds **9d** and **10d**) were more effective than at *meta* position (compounds **9e** and **10e**).

In the antifungal assay of synthetic compounds against *R. solani*, compounds **7a**, **7b** and **9d** were found to be the most effective antifungal compounds, which showed similar or better antifungal activity than their parent compounds and chlorothalonil. The compounds with different heterocyclic unit, terpene structure and substituent showed different antifungal activity, but there was no significant regular change. Compound **9d** and **9e** were the only two compounds which exhibited some antifungal activity against *P. oryzae* in this study. All the other compounds, however, were less effective. Thus, we cannot yet draw a clear conclusion about the structure-activity relationships of test compounds against *R. solani* and *P. oryzae* according to the current test results.

It is not the first time the hybrid molecules of carvacrol and thymol were synthesized. Recently, some hybrid molecules were designed through introducing different active structures to carvacrol and thymol molecules. For example, hybrid molecules of carvacrol and sulphur-containing amino acids revealed good antifungal activity against *Candida albicans* (Cacciatore et al. 2015). Bendre and co-workers (Pete et al. 2012) synthesized benzoyl phenyl urea derivatives with the structure of carvacrol, and several compounds have exhibited application possibility in agriculture and medicine. Srinivas and coworkers (James Bound et al. 2016) synthesized a series of novel 2,3-unsaturated and 2,3-dideoxy 1-*O*-glucosides of carvacrol, thymol, and perillyl alcohol, the 2,3-dideoxyglucosides of carvacrol and thymol showed high antifungal activity and could potentially be used as antifungal agents. And in this study, we synthesized some carvacrol and thymol esters and the antifungal activity *in vitro* of the most effective compound (compound **9d**) was close to chlorothalonil against both *B. cinerea*, *P. oryzae* and *R. solani*. In previous studies, carvacrol and thymol ester derivatives with structure of 5-phenyl-2-furan were synthesized and their antifungal activity was evaluated, and the results showed that most of the title compounds had a considerable effect on the selected plant pathogenic fungi (Cui et al. 2014). In our study, our primary concern was that the influence of introducing different heterocyclic units in carvacrol and thymol esters, and heteroaromatic carboxylic acids without substitute was chemically combined with the carvacrol and thymol molecules. Fortunately, some hybrid molecules also exhibited excellent antifungal activity, which were higher than previously reported carvacrol

or thymol esters. Nevertheless, more picolinate derivatives should be synthesized to get more effective antifungal ingredient for our further research.

3. Experimental section

3.1. General experimental

All of the reagents and solvents for reaction were purchased from Aladdin (China) or Sinopharm Chemical Reagent Co., Ltd (China) were of analytical grades and used without further treatments. Solvents for extraction and chromatography were of technical grade and distilled prior to use. Reactions were monitored by thin layer chromatography (TLC) by using silica gel coated glass slides (silica gel 60 GF 254, Qingdao Haiyang Chemical, China). Detections were conducted under UV (254 nm). ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance III 500 NMR spectrometer. The chemical shifts (δ) were reported in ppm with reference to internal TMS, and coupling constants (J) were given in Hz. ESI-MS spectra were recorded on a Thermo Fisher Scientific TSQ Endura MS.

3.2. Chemistry

3.2.1. 4-Bromocarvacrol (3)

To a solution of carvacrol (1.5 g) in glacial acetic acid (10 mL) cooled to 0 °C was added Br_2 (0.51 mL) over 20 min, then the reaction mixture was stirred for 3 h at room temperature. The mixture was poured out on ice water (20 mL), and the aqueous solution was extracted with dichloromethane (3 × 10 mL). The combined organic phase was dried over anhydrous Na_2SO_4, filtered, and the solvent was removed *in vacuo* to give a yellow oil. The oil was purified by column chromatography using silica gel with PE/EA (40:1, v/v) as eluent to afford compound 3.

Compound 4 was prepared as described for compound **3**.

3.2.2. 5-isopropyl-2-methylphenyl furan-2-carboxylate (7a)

A solution of furan-2-carboxylic acid (1.2 mmol, 1.2 equiv) in thionyl chloride (1 mL) was refluxed for 3 h, then thionyl chloride was removed *in vacuo*. The residues were dissolved in anhydrous THF (1 mL) and was added to a solution of carvacrol (1 mmol, 1.0 equiv) and triethylamine (1.5 mmol, 1.5 equiv) in anhydrous THF (4 mL). The mixture was stirred for 1 h at room temperature. After the reaction was completed, 5 mL of water was added to the reaction mixture, then the reaction mixture was extracted with EtOAC (2 × 10 mL). The organic layer was dried over anhydrous Na_2SO_4. The solvent was removed *in vacuo*, and the oil was purified by silica gel colunm chromatography (Petroleum ether : EtOAC = 20:1) to give compound **7a**.

Compounds **7b-d**, **8a-d**, **9a-f** and **10a-f** were prepared as described for compound **7a**.

3.3. Antifungal assays

The *in vitro* antifungal activity of target compounds was determined by the mycelium growth rate method. Five kinds of important plant pathogenic fungi (*Alternaria solani*, *Botrytis cinerea*, *Fusariu oxysporum*, *Pyricularia oryzae*, and *Rhizoctonia solani*) were chosen for

antifungal assay. The experimental details and computing methods are the same as we described in our recent paper (Wang et al. 2018).

4. Conclusions

In conclusion, twenty carvacrol and thymol esters with heteroaromatic carboxylic acids were synthesized, and their antifungal activity was evaluated against five phytopathogens. The results showed that some synthetic compounds exhibited higher antifungal activity than their precursor compounds and commercial chlorothalonil, and compound **9d** (*4-bromo-5-isopropyl-2-methylphenyl picolinate*) was the most potential antifungal agent in this study, which exhibited a broad antifungal spectrum against *B. cinerea*, *P. oryzae* and *R. Solani*. The introduction of different heterocyclic units in the carvacrol and thymol molecules could influence their antifungal activity, carvacrol and thymol esters with furan, thiophene and pyridine unit exhibited good antifungal activity and a bromine atom on the *para* position of pyridine unit can help to enhance their antifungal effect. We are in the process of additional antifungal assay to investigate the bioactivity of compound **9d**. And for our further work, more picolinate derivatives should be synthesized.

Supplementary material

Spectral data of all synthetic compounds and experimental data of antifungal assay relating to this article can be found in the online versions as Appendix.

Disclosure statement

No potential conflict of interest was reported by the authors.

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