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Synthesis and biological evaluation of novel trichodermin derivatives as antifungal agents

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ABSTRACT

To discover more potential antifungal agents, 17 novel trichodermin derivatives were designed and synthesized by modification of **3** and **4a**. The structures of all the synthesized compounds were confirmed by ^{1}H NMR, ESI-MS and HRMS. Their antifungal activities against *Ustilaginoidea oryzae* and *Pyricularia oryzae* were evaluated. Most of the target compounds showed potent inhibitory activity, in which **4g** showed superior inhibitory effects than **4a** and commercial fungicide prochloraz. Furthermore, **4h** demonstrated comparable inhibitory activity to **4a**. Moreover, **4i** and **4l** exhibited excellent inhibitory activity for *Pyricularia oryzae*. Additionally, compound **9** was found to be more active against all tested fungal strains than **3**, with EC_{50} values of 0.47 and 3.71 mg L^{-1}, respectively.

Fungal diseases have long been one of the major causes of crop losses, and to control the epidemic of these diseases has a vital importance.[1] To date, the commonly treatment method for the epidemic spread of fungal diseases is the application of chemical fungicide. The excessive use of these synthetic chemicals over the years, however, has led to the rapid development of fungicide resistance, which in turn makes the management of fungal diseases more precarious.[2–4] Therefore, there is an urgent need for the development of novel, effective and environmentally-friendly agents to replace the conventional chemical fungicides.

Natural products have received a considerable attention as shown by the numerous studies published on their broad-spectrum biological activities, such as insecticidal, anti-inflammatory, antifungal, antitumor and antibacterial activities.[5–11] Trichodermin (**1**, Fig. 1), a naturally occurring sesquiterpene antibiotic, was isolated from metabolites of fungi, which had been found to possess excellent antifungal activity.[12,13] Trichodermin was considered as one of the ideal lead compounds for new fungicides because of its unique mechanism of action that could inhibit protein synthesis in eukaryotes.[14–16]

As far as structure-activity relationship is concerned, in our previous studies, series of structure modifications on trichodermin mother nucleus have been carried out, indicating that appropriate substituents introduced to the C-4 and C-8 position could help improve the antifungal activities of the compounds, and the conjugated structure in molecular could contribute to enhancing their bioactivity.[17,18] Among these compounds, **3** and **4a**, which all contained conjugated structure, exhibited relatively stronger antifungal activities against *Ustilaginoidea oryzae* (*U. ory.*) and *Pyricularia oryzae* (*P. ory.*) than trichodermin (Fig. 1). Therefore, inspired by the above results, we designed and synthesized a series of novel trichodermin derivatives based on the general structures of **3** and **4a** to better understand the structure–activity relationship.

The synthesis of the key intermediates **2** and **3** were readily obtained in one step from starting material trichodermin. The synthetic pathway was depicted in Scheme 1. In our previous manuscript, we disclosed a method to obtain **2** and **3** by reacting **1** with stannic oxide in 1,4-dioxane.[18] Through this method, **2** could be obtained as expected. However, intermediate **3** as by-product resulted in a relatively low yield. To optimize this reaction, several different solvent systems were investigated (Table 1). According to the screening of solvent systems, acetonitrile/H_2O (10:1, v/v) (Table 1, entry 3) presented a relatively higher yield of both **2** and **3**, which was then introduced to produce these two key intermediates.

In an attempt to explore the electronic effect of the substituent in the benzene ring on the investigated biological activity, the desired compounds **4a**–**4m** were prepared through treating **2** with substituted cinnamoyl chloride in the presence of triethylamine as acid acceptor and *N*,*N*-dimethylaminopyridine as catalyst, as shown in Scheme 2. In addition, considering that the chirality of drug isomer sometimes has significant effects on their biological activity,[19,20] we designed **4n** (cis-conformer of **4a**) via the route outlined in Scheme 2. On the other hand, we also did some proper structure modification on **3** with instability of aldehyde group taken into account. As outlined in Scheme 3, thiazole amine, which

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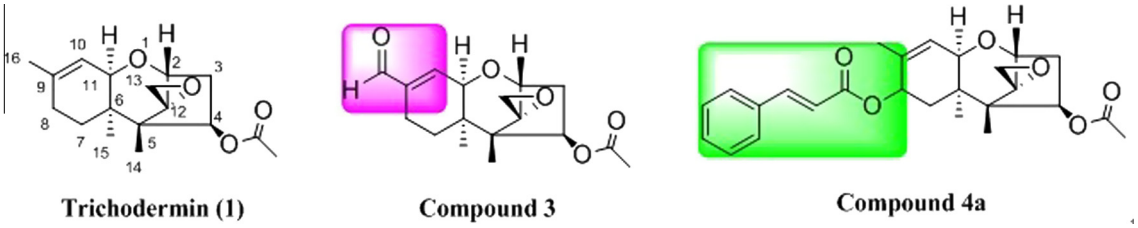
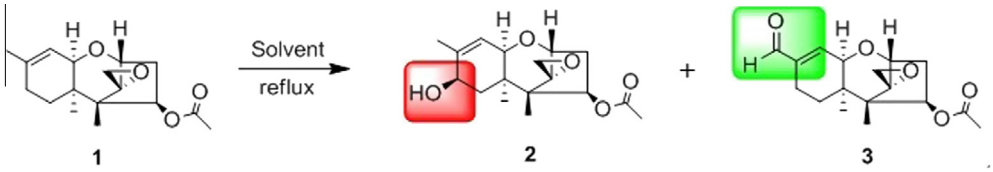


Figure 1. Chemical structures of trichodermin (**1**), compounds **3** and **4a**.



Scheme 1. Synthetic route to compounds **2** and **3**.

Table 1
The optimization of compounds **2** and **3**

Entry	Solvent	Yield (%)	
		2	**3**
1	1,4-Dioxane/H_2O (10:1, v/v)	50	25
2	1,4-Dioxane /TBHP[a] (10:1, v/v)	32	15
3	Acetonitrile/H_2O (10:1, v/v)	50	35
4	Acetonitrile/TBHP (10:1, v/v)	45	20
5	Dicloromethane/TBHP (10:1, v/v)	36	22
6	Ethanol (95%)	26	12
7	THF[b]	70	5
8	Xylene	56	2
9	Acetone	30	1

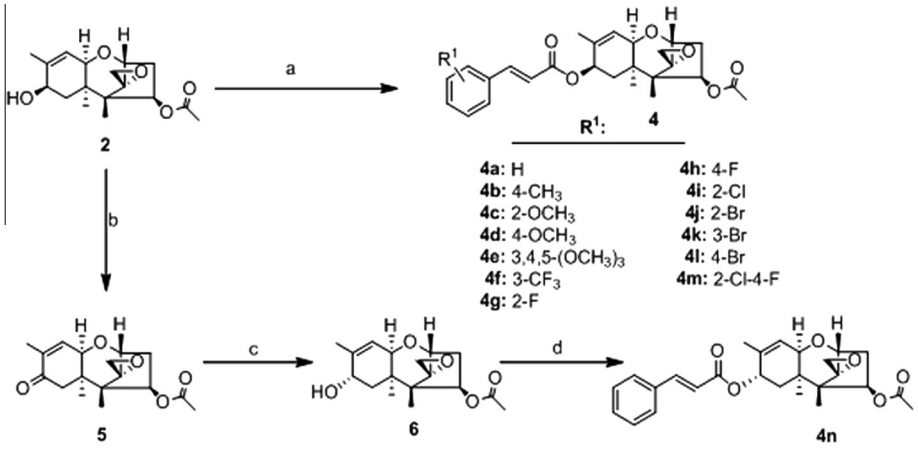
[a] TBHP: *tert*-butyl hydroperoxide.
[b] THF: tetrahydrofuran.

is usually used as antifungal pharmacophore, was reacted with **3** in dry methanol to give compounds **7a** and **7b**. Compound **8** was prepared by treatment of **3** with potassium carbonate, which reacted further with acetyl chloride to yield **9** (to improve its lipophilicity). The structures of all the synthesized compounds were confirmed by ^{1}H NMR, ESI-MS and HRMS analyses.

In our previous study, the 3D structural information of compound **4a** was confirmed by single-crystal X-ray diffraction analysis.[18] In this Letter, in order to identify the stereostructure of **3**, we were successful in preparing X-ray quality single crystal of intermediate **3** obtained by slow evaporation of its 95% ethanol solution (CCDC 934209). As shown in Figure 2, the crystal was monoclinic with space group *P212121*, and the cell parameters were $a = 7.1994(3)$ Å, $b = 12.9564(5)$ Å, $c = 16.9369(7)$ Å, $V = 1579.85(11)$ Å^3, $Z = 4$. The final $R = 0.0398$, $wR = 0.0987$, $S = 1.000$, the difference density were <0.001 e/Å^3 (max) and −0.145 e/Å^3 (min).

The in vitro antifungal activities of all the target compounds have been evaluated against two representative pathogenic fungi including *U. ory.* (ACCC 36443) and *P. ory.* (ACCC 37631) by the mycelium growth rate method.[21,22] Commercial fungicide prochloraz was used as a comparative control. Each treatment was performed three times, and the effective concentration 50 (EC_{50}) defined as the concentration required to inhibit 50% of the fungal growth, was used to describe the inhibitory activity of the compounds. The antifungal activities of the target compounds along with the standard drug for comparison are summarized in Tables 2 and 3.

Initially, we aimed to investigate the influence of substituent in the benzene ring on the antifungal activity. From the data presented in Table 2, we can see that when **4a** was modified by varying the substituents on the benzene ring, the antifungal activities of these target compounds were changed obviously. When *para*-position of the benzene ring of **4a** was substituted with a



Scheme 2. Reagents and conditions: (a) Substituted cinnamoyl chloride, Et_3N, DMAP, CH_2Cl_2, rt, 75–85%; (b) CrO_3, pyridine, 80 °C, 3 h, 68%; (c) KBH_4, NH_4Cl, H_2O, CH_3OH, 24 h, rt, 45%; (c) Cinnamoyl chloride, Et_3N, DMAP, CH_2Cl_2, rt, 12 h, 84%.