SHORT COMMUNICATION

Natural products as sources of new fungicides (II): antiphytopathogenic activity of 2,4-dihydroxyphenyl ethanone derivatives

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A series of 17 simple 1-(2,4-dihydroxyphenyl) ethanones were synthesised, and their structures characterised by $^1$H, $^{13}$C NMR and ESI-MS. Their in vitro antifungal activities were evaluated against five phytopathogenic fungi including Glomerella cingulate, Botrytis cinerea, Fusarium graminearum, Curvularia lunata and Fusarium oxysporum f. sp. vasinfectum by the mycelial growth inhibition assay. Compounds 2g and 2h exhibited broad-spectrum inhibitory activity against the mycelial growth of the tested pathogens with IC$_{50}$ values in the range of 16–36 $\mu$g/mL, and in particular being more active to G. cingulate, with IC$_{50}$ values of 16.50 and 19.25 $\mu$g/mL, respectively, than the other pathogens. Preliminary SAR indicated that an $\alpha,\beta$-unsaturated ketone unit of the alkyl chain of the compounds is the structure requirement for fungicidal action. The results suggested that 2g and 2h may be promising leads in the development of new antifungal agents.

Keywords: acetophenones; benzophenones; phytopathogenic fungi; antifungal activity; structure–activity relationship

1. Introduction

Agricultural crops are facing tremendous losses due to pests, diseases and weed damages which result in direct economic losses including reduction in grain yield and quality (Thind 2012; Madrid Villegas et al. 2015). Despite the availability of effective synthetic fungicides, new fungicides with novel chemical structures which have higher potency and broader spectrum of activity against resistant fungal strains are still needed. Recent studies have demonstrated that acetophenone and its derivatives can be seen as important intermediates for the synthesis of some agrochemicals and pharmaceuticals. For example, two antifungal agents 1-(2-hydroxy-4,6-dimethoxy) acetophenone (xanthoxylin) from a medicinal plant Melicope borbonica (Simonsen et al. 2004) and 2,4-dihydroxy-5-methylacetophenone from the fungus Polyporus picipes (Bai et al. 2013; Ma et al. 2013). Based on these antecedents, the aim of this work was to...
prepare a series of 2,4-dihydroxyphenyl ethanones: 2a–n, 3, 4 and 5, and evaluated \textit{in vitro} antifungal activities against several fungal phytopathogens. Herein, we report the interesting findings of these simple acetophenone derivatives.

2. Results and discussion

The synthetic pathways for the preparation of 2,4-dihydroxyacetophenone analogues 2a–n are shown in Figure 1. Target compounds 2a–n could be readily obtained in a single step by the Fries rearrangement of the appropriate phenol esters (method B) or by the Friedel–Crafts acylation reaction of resorcinol with the proper acyl chlorides in the presence of dry AlCl$_3$ in good yields (method A). Specifically, as stated in the Experimental Section (Supplementary Material), benzoylated and some alkanoylated products with a large steric hindrance group, such as 2c, 2k and 2m, were obtained by increasing reaction temperature and time in order to get a reasonable yield, and the best result was obtained for 2c at 100°C for 3 h in yield of 82%. In addition, the preparation of compounds 3 (93 mg, 56%), 4 (139 mg, 90%) and 5 (135 mg, 81%) started with 2a following the conventional methods (Figure 2). The spectroscopic data of the semi-synthetic compounds are given in Supplementary Material.

![Figure 1. Synthesis of compounds 2a–n.](image1)

![Figure 2. Synthesis of compounds 3–5.](image2)
Preliminary *in vitro* screening results of the title compounds for antifungal activities against five phytopathogenic fungi (*Glomerella cingulate*, *Botrytis cinerea*, *Fusarium graminearum*, *Curvularia lunata* and *Fusarium oxysporum* f. sp. *vasinfectum*) are listed in Table S1 (Li et al. 2012; Lu et al. 2014). The results indicated that some of the synthetic compounds exhibited over 65% growth inhibition against mycelial growth of these tested fungi at concentrations of 100 μg/mL. As shown in Table S1, among the semisynthetic derivatives, compounds 2a–d and 2f, with a saturated aliphatic chain C1–C4, showed weak antifungal activities against the test pathogenic fungi with <65% inhibition (except *B. cinerea*), while 2e, with a saturated aliphatic straight C4 chain exerted strong activities against *G. cingulate*, *B. cinerea*, *F. graminearum* and *C. lunata*, with the inhibition of 68.75%, 74.52%, 65.50% and 76.83%, respectively. This indicated that the alkyl chain length appeared to be required for activity. The introduction of the unsaturated aliphatic carbon chain into 1 gave compounds 2g and 2h, producing more increased activity compared to 2a–e and 2j. They almost completely inhibited the mycelial growth of the pathogens with 92% to 98% inhibition (Table S1). Both 2g and 2h were significantly more active against *G. cingulate* and *C. lunata* than a commercial fungicide thiabendazole as positive control. The results suggested that the presence of a conjugated (−CH=CH−C−O−) unit in the alkyl chain plays an important role in the activity. In addition, 2i with a chlorine atom in the side chain was better than 2a in activity, implying that the Cl group is more sensitive to the tested pathogens. Furthermore, the introduction of mono-substituted aromatic rings decreased activity of benzophenones (2k–m) with 7% to 58% inhibition, possibly due to a steric hindrance. Also, among the tested compounds 3–5, 4 and 5 reduced the mycelial growth of *B. cinerea* up to 73.35% and 75.45% at 100 μg/mL, respectively, but were inactive to other pathogens tested. The results suggested that the OH group at C-4 is very sensitive to *B. cinerea* (3 vs 5).

Compared to other tested compounds, the highest activities with broad antifungal spectra of 2g and 2h make them good candidates for further evaluation, and their median inhibitory concentration (IC50) was thus determined (Li et al. 2012). The IC50 values for 2g and 2h against *G. cingulate* were 16.50 and 19.25 μg/mL, respectively; a slightly higher IC50 values (2g, 22.55 μg/mL; 2h, 24.52 μg/mL) were found against *F. oxysporum* f. sp. *Vasinfectum*, whereas *F. graminearum* Sehw. was found less sensitive (IC50 values of 25.62 and 27.49 μg/mL, respectively). Moreover, *B. cinerea* and *C. lunata* were weak sensitive organisms, with IC50 values of 32.05 and 35.37 μg/mL for 2g and 2h against *B. cinerea* and of 34.37 and 36.52 μg/mL against *C. lunata*. These results confirm that the co-occurrence of acidic phenolic hydroxyls and lipophilic residues is an important chemical feature for antifungal activity. Taken together, the order of their antifungal potency is R = allylic group > -chloromethyl > alkanyl ≫ aromatic group. This suggests that the addition of the aliphatic side chain increases the lipophilicity of the compound resulting in a greater antifungal activity, which might be associated with better penetration through the plasmatic membrane of the fungi.

3. Conclusions
In conclusion, a series of acetophenone derivatives were prepared and their *in vitro* antifungal activities against five phytopathogens were evaluated. Among the synthetic derivatives, compounds 2g and 2h displayed broad-spectrum antifungal activities to various important plant pathogens and might be promising leads for new agricultural fungicides.

Supplementary material
Supplementary material relating to this article is available online, alongside Table S1.
Disclosure statement
No potential conflict of interest was reported by the authors.

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