Antifungal and insecticidal activities of rhein derivatives: synthesis, characterization and preliminary structure–activity relationship studies

Xiang Zhu\textsuperscript{a,b,*}, Shunshun Chen\textsuperscript{a,b,*}, Yan Zheng\textsuperscript{a,b}, Yong Zhang\textsuperscript{a,b}, Tom Hsiang\textsuperscript{c}, Rong Huang\textsuperscript{a,b}, Jingwei Qi\textsuperscript{a,d}, Tian Gan\textsuperscript{a,b}, Yue Chang\textsuperscript{a,b} and Junkai Li\textsuperscript{a,b}

\textsuperscript{a}Hubei Engineering Technology Center for Pest Forewarning and Management, College of Agriculture, Yangtze University, Jingzhou, China; \textsuperscript{b}Institute of Pesticides, Yangtze University, Jingzhou, China; \textsuperscript{c}School of Environmental Sciences, University of Guelph, Guelph, ON, Canada; \textsuperscript{d}Institute of Entomology, College of Agriculture, Yangtze University, Jingzhou, China

ABSTRACT
There is an urgent need to replace highly polluting pesticides with environmentally friendly green pesticides of high efficiency and low toxicity, because of the growing concern for quality and safety of agricultural products. To discover new pesticides with diverse chemical structures from natural products, a series of rhein derivatives \textsuperscript{3a–9b} were designed, synthesized, and evaluated for their antifungal activity and insecticidal activity. The bioassay showed that some compounds exhibited moderate antifungal activity against \textit{Rhizoctonia solani}, but lower activity against the other five pathogens. Surprisingly, most compounds displayed potent insecticidal activity against \textit{Spodoptera litura} and \textit{Tetranychus cinnabarinus} at a concentration of 2 \(\mu\text{mol/mL}\). In particular, compounds \textsuperscript{3a, 5a, and 3b} exhibited potent insecticidal activities against \textit{S. litura} at 72 h, with mortality rates of 100%, 100% and 92.1%, respectively, which were equivalent to that of the insecticide fipronil (100%). Their structure–activity relationships were also discussed. The findings of this experiment provide helpful research ideas for the development of these rhein derivatives as novel natural product-based pesticides in crop protection.

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1. Introduction

Pesticides are needed to ensure agricultural production, and their role in agricultural modernization cannot be replaced. Countries around the world are increasingly advocating environmental protection and sustainable development (Zhang et al. 2018; Zhu et al. 2018; Wang et al. 2019; Yu et al. 2019; Wu et al. 2021), and the public pays more attention to the quality and safety of agricultural products, their own health and the health of the environment with higher expectations (Niu et al. 2016; Lv et al. 2018; Yang et al. 2020). However, while pesticides play an important role, they have also attracted criticism and attention because of potential side effects including risks to human health and the environment. Therefore, there is a need to find environmentally friendly pesticides with high efficiency and low toxicity (Xiong et al. 2017; Bai et al. 2019; Zhu et al. 2019; Tantawy et al. 2020).

Natural products (NPs), including semi-synthetic NPs and NP-derived compounds, with a high degree of chemical diversity and biochemical specificity, have a long history as active ingredients and inspiration for a wide range of pharmaceutical and crop protection compounds (Yang et al. 2015; Yu et al. 2018; Xiong et al. 2019; Han et al. 2020). It is well-known that using natural products as the lead compound is an effective method for the discovery of environmentally friendly green pesticides (Huang et al. 2018; Lorsbach et al. 2019). The 1,8-dihydroxy anthraquinones are essential structural motifs in a great number of plant-derived natural products such as rhein, emodin, aloe-emodin and physcion (Figure 1) (Agarwal et al. 2000). These compounds which were isolated or derived from the plant Rheum palmatum L. have exhibited extensive biological properties, such as anticancer activity, anti-inflammatory activity, antiviral activity, among others (Yang et al. 2011; Suneela & Dipmala 2012; Liang et al. 2014; Xu et al. 2016). Therefore, we tested the antifungal and insecticidal activities of the four natural products mentioned above, and we had the preliminary results summarized in Table S4 (supplementary material) suggesting that rhein was suitable as a lead compound for the creation of new green pesticides.

In continuation of our efforts aimed at discovery of new natural-product-based environmentally friendly green pesticides, 17 rhein derivatives, 3a–9b were synthesized by derivatization of the 1, 8-position hydroxyl group and carboxyl group of rhein in consideration of its structural feature. The antifungal activity of these compounds
was assayed against six important phytopathogenic fungi including *Rhizoctonia solani*, *Sclerotinia sclerotiorum*, *Fusarium graminearum*, *Fusarium pseudograminearum*, *Bipolaris maydis*, and *Phytophthora capsici*. Also, the insecticidal activity was investigated against the *Spodoptera litura* and *Tetranychus cinnabarinus*. This type of work on structure and discovery may provide new insights into the molecular design of rhein analogues as crop protection agents.

2. Results and discussion

2.1. Chemistry

The structure and synthesis route of rhein derivatives are shown in Scheme 1 (Viayna et al. 2014). Treatment of Rhein with at reflux temperature in CH$_2$Cl$_2$ solution afforded

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**Scheme 1.** Synthesis of rhein derivatives (3a–9b). Reagents and conditions: (a) SOCl$_2$, CH$_2$Cl$_2$, DMF, reflux, 8 h; (b) Amine, CH$_2$Cl$_2$, room temperature, 2~3 h; (c) Alcohol, Et$_3$N, room temperature, 2~3 h; (d) Acid chloride, CH$_2$Cl$_2$, Et$_3$N, room temperature, 1 h; (e) CH$_3$I, NaH, DMF, 0 °C, to reflux, 3 h; (f) NaOH, CH$_3$OH-H$_2$O (1:1), room temperature, 2 h; (g) SOCl$_2$, CH$_2$Cl$_2$, DMF, reflux, 8 h, then, CH$_2$Cl$_2$, Et$_3$N, 0 °C to room temperature, 2 h; (h) LiOH, CH$_3$OH-H$_2$O (1:1), room temperature, 2 h.
intermediate 2 after the evaporation of the solvent. Subjecting intermediate 2 to corresponding amine led to rhein amides 3a–3d and to the alcohols led to rhein esters 4a–4d. Then, Rhein methyl ester 4a was used as the substrate to react the phenolic hydroxyl group on its structure with acyl chloride to obtain rhein ester derivatives 5a and 5b. Methoxilation of Rhein esters 4a and 4b with methyl iodide afforded rhein derivatives 6a and 6b. Methyl ether rhein derivative 7 was obtained by hydrolysis of 6a obtained in the previous step with sodium hydroxide. In the synthesis of rhein amino acid ester derivatives 8a and 8b, methyl ether rhein 7 was first prepared into formyl chloride with SOCl₂ and then coupled with amino acid ester. The rhein amino acid derivatives 9a and 9b were prepared by hydrolyzing the corresponding rhein amino acid esters derivatives 8a and 8b with lithium hydroxide. The structures of all synthesized derivatives were well characterized by 1H NMR, 13C NMR and HRMS analyses (The corresponding spectra were given in the Supporting Information).

2.2. Antifungal activity
All the synthesized compounds were primarily evaluated for their in vitro antifungal activity against six common phytopathogenic fungi, *Rhizoctonia solani*, *Sclerotinia sclerotiorum*, *Fusarium graminearum*, *Fusarium pseudograminearum*, *Bipolaris maydis*, and *Phytophthora capsici*, each at a concentration of 0.2 μmol/mL (Zhu et al. 2019; Hao et al. 2020). The results of in vitro antifungal activity are shown in Table S1 (supplementary material). Results indicated that, the target compounds showed different levels of antifungal activity. They showed higher inhibitory activities against *R. solani*, some higher than the parent compound rhein. However, the target compounds had lower activity against the other five pathogens. Among them, only compound 3a exhibited more than 50% inhibitory activity against *R. solani*, *S. sclerotiorum* and *P. capsici*. From the preliminary structure–activity relationship (SAR) of the target compound, it was found that the antifungal activity of the rhein amide is higher than that of the rhein ester. That is to say, derivatizing the carboxyl group of rhein into an amide group increased its antifungal activity. The antifungal activity of amide derivatives 3a–3d was arranged in the order of 3a >3b >3c >3c, indicating that the R¹ as alkyl group was more active than that of aryl group substitution. When the hydroxyl group of rhein was esterified (5a and 5b) or alkoxylated (6a and 6b), its antifungal activity was lower, which indicated that the hydroxyl group of rhein should be completely retained. On the whole, current rhein derivatives showed low antifungal activity and are not suitable for use as agricultural fungicides.

2.3. Insecticidal activity
2.3.1. Acaricidal activity against tetranychus cinnabarinus
The acaricidal activity of compounds against adult *T. cinnabarinus* was tested by leaf-dipping method (Hao et al. 2020). Commercial pyridaben was used as a positive control. The corrected mortality rates at 24 h, 48 h and 72 h of the tested compounds at a concentration of 2 μmol/mL are shown in Table S2 (supplementary material). It can be seen that all target compounds showed some acaricidal activity against adult *T.
cinnabarinus, but all lower than commercial acaricide, pyridaben. As the feeding time increased, the rhein derivatives showed higher mortality rates to T. cinnabarinus, with some compounds showing the mortality rates of more than 50% (3a, 9a, and 9b). Particularly, the rhein amino acid derivatives 9b and 9a showed best the best acaridal activities against T. cinnabarinus with the mortality rates of 66.2 and 57.3% respectively. Structure-activity relationship analysis revealed that the acaricidal activity of the rhein derivatives with R1 or R2 = alkyl groups were higher than that of compounds with R1 or R2 = aryl groups. For example, compounds 3a and 3b displayed higher acaricidal activities than that of compound 3c and 3d. In addition, the acaricidal activity decreased significantly when the hydroxyl group of rhein was derived to an ester group (5a, 5b) or alkoxy group (6a, 6b), indicating that the hydroxyl group should be retained and is the active moiety of rhein. Interestingly, after introducing amino acids into the structure, the two rhein amino acid derivatives 9a and 9b showed the highest acaricidal activity, which provides useful research ideas for the design of new members of these type derivatives as well as for future biological studies.

2.3.2. Insecticidal activity against Spodoptera litura
The insecticidal activity of compounds against third-instar larvae of S. litura were tested using the leaf-dipping method (Huang et al. 2014), at a dosage of 2 μmol/mL, and a commercial insecticide fipronil was used as positive control at the same concentration. The insecticidal activity against S. litura is shown in Table S3 (supplementary material). The results indicated that most of the compounds showed different levels of insecticidal activity against S. litura at a concentration of 2 μmol/mL. At 24 h, the mortality rates using rhein derivatives against S. litura were generally low, among which the highest was 35.6% for compound 4a, but much lower than 88.9% for the control insecticide fipronil. As the feeding time increased, the mortality using rhein derivatives on S. litura increased gradually, and compounds 4a, 3b, 3c and 5a exhibited potent insecticidal activities against S. litura at 48 h, with the mortality rates of 91.8%, 77.6%, 67.4% and 62.9%, respectively. In particular, the mortality rates at 72 h of 3a, 5a and 3b were 100%, 100% and 92.1%, respectively, which were not significantly different than that of fipronil (100%). For compounds 4a–4d, it was noteworthy that the mortality rates caused by rhein esters compounds 4a and 4b with R2 = alkyl groups were evidently higher than that of compounds 4c and 4d with R2 = aryl groups. Similar results were also observed in rhein amides compounds 3a and 3b, which indicated that the introduction of alkyl group increased insecticidal activity against S. litura. The rhein amino acid derivatives 9a and 9b exhibited more potent activity than their corresponding amino acid ester derivatives 8a and 8b. This suggested that the introduction of amino acid moiety of rhein should be a target for future modification and testing.

3. Conclusions
In summary, in exploration of new natural product-based environmentally friendly green pesticides, a series of rhein derivatives 3a–9b were synthesized, and their structures were characterized and confirmed by 1H NMR, 13C NMR and HRMS analyses. The antifungal activity against six important phytopathogenic fungi and insecticidal activity
against Spodoptera litura and Tetranychus cinnabarinus of these rhein derivatives were evaluated. The bioassay showed that some compounds exhibited moderate antifungal activity against R.solani, but lower activity against the other five pathogens. Surprisingly, at a dosage of 2 μmol/mL, most rhein derivatives displayed insecticidal activity against S.litura and T.cinnabarinus, and some of them showed potent insecticidal activities. Especially, the rhein amino acid derivatives 9b and 9a showing the highest acaricidal activities against T. cinnabarinus at 72 h with the mortality rates of 66.2 and 57.3% respectively. Compounds 3a, 5a and 3b exhibited potent insecticidal activities against S. litura at 72 h, with the mortality rates of 100%, 100% and 92.1%, respectively, which were similar to that of fipronil (100%). The results on antifungal and insecticidal activities study of rhein derivatives provide helpful research ideas for the development of such derivatives as novel natural product-based pesticides in crop protection, with specific suggestions for future research.

Disclosure statement
No potential conflict of interest was reported by the authors.

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