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Bioactivity and structure–activity relationship of cinnamic acid derivatives and its heteroaromatic ring analogues as potential high-efficient acaricides against *Psoroptes cuniculi*

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ABSTRACT

A series of cinnamic acid derivatives and its heteroaromatic ring analogues were synthesized and evaluated for acaricidal activity in vitro against *Psoroptes cuniculi*, a mange mite. Among them, eight compounds showed the higher activity with median lethal concentrations (LC_{50}) of 0.36–1.07 mM (60.4–192.1 μg/mL) and great potential for the development of novel acaricidal agent. Compound **40** showed both the lowest LC_{50} value of 0.36 mM (60.4 μg/mL) and the smallest median lethal time (LT_{50}) of 2.6 h at 4.5 mM, comparable with ivermectin [LC_{50} = 0.28 mM (247.4 μg/mL), LT_{50} = 8.9 h], an acaricidal drug standard. SAR analysis showed that the carbonyl group is crucial for the activity. The type and chain length of the alkoxy in the ester moiety and the steric hindrance near the ester group significantly influence the activity. The esters were more active than the corresponding thiol esters, amides, ketones or acids. Replacement of the phenyl group of cinnamic esters with α-pyridyl or α-furanyl significantly increase the activity. Thus, a series of cinnamic esters and its heteroaromatic ring analogues with excellent acaricidal activity emerged.

Animal acariasis is a skin disease caused by mites, which are widely parasitic on the body surfaces or epidermises of animals or human. *Psoroptes cuniculi* is an animal ear mite living in the ear canal of animals and can infect rabbits, goats, horses, buffalo, sheep and so on.[1] Additionally, *Psoroptic* acariasis is a highly contagious disease. The infestation can cause intense pruritus, inflammation, serous exudations, the formation of crusts and scabs, reduction of weight gain, and even death.[2] At present, psoroptic acariasis has been a global disease, which causes serious economic losses for the animal industry.[3]

Therapy and control of both human scabies and animal mange mainly depend on the use of drugs and chemicals.[4] Among them, organochlorine, organophosphates, pyrethrins,[5] especially, ivermectin (Fig. 1) and abamectin[6] have been more commonly used drugs for treatment and control of animal acariasis. However, continuous use of these drugs has presented some serious problems such as drug-resistance,[7,8] toxicity and environmental damage.[9] Therefore, it is urgent to develop new effective and safe acaricidal agents for treatment and control of animal acariasis.

Over the past decades, cinnamic acid and its esters widely distributed in plants[10] have attracted much attention of many pharmacologists due to a variety of important pharmacological activities[11] such as anticancer,[12] antimicrobial,[13] antioxidative,[14] anti-inflammatory,[15] anti-Mycobacterium tuberculosis,[16] antiviral,[17] anti-human immunodeficiency virus (HIV),[18] antidiabetic,[19] anticholesterolemic,[20] hepatoprotective,[21] immunoprotective,[22] inducing neural progenitor cell proliferation[23] and anti-parasitic activities.[24] Additionally, cinnamic acid and its esters also have low toxicity to mammals.[25] Thus, cinnamic acid derivatives are already a class of very promising lead compounds for the development of new, highly effective drugs.

Our previous research found that ethyl cinnamate derivatives with substituents on the benzene ring are a class of promising lead compounds for the development of new acaricidal agents against *P. cuniculi*.[26] The structure-activity relationship showed that the substitution pattern on the benzene dramatically impact the activity. Strong electron-withdrawing groups like *o*-NO_2 or *m*-NO_2 can significantly improve the activity whereas hydroxy, methoxy, acetoxy, methylenedioxy, bromo or chloro groups led to decrease of the activity. As our continuing research, in order to more fully know the SAR of cinnamic acid esters and discover more potent acaricidal compounds, in the present study, a series of cinnamic acid derivatives including esters, amides, thiol esters, ketones,

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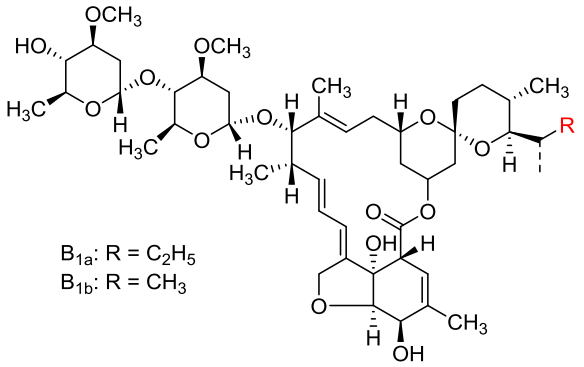

Fig. 1. Structure of ivermectin (the mixture B_{1a} and B_{1b}).

ethers and its heteroaromatic ring analogues were prepared and evaluated for acaricidal activity against *P. cuniculi*. Meanwhile, the structure-activity relationship was discussed also.

In order to have an insight into the effect of the chain length, branching degree and type of the alkyl group in the ester moiety on the activity, a series of cinnamic acid esters (**1**–**23**) formed from cinnamic acid and various alcohols or phenols were designed (Table 1). The alkyl groups include C1-C8 linear alkyl, C3-C5 branched-chain alkyl, cyclohexyl, benzyl and substituted benzyl, phenyl and substituted phenyl. Additionally, we also designed some amide, thioester, ketone or ether derivatives (**24**–**30**) (Table 1) of ethyl cinnamate to inspect the relationship between the ester group itself or its two oxygen atoms and the activity. On the other hand, a series of heteroacromatic ring analogues (**32**–**45**) (Table 1) were also designed to know the influence of various heteroaromatic rings on the activity. It was expected that the structural similarity and diversity could lead to the discovery of more potent acaricidal compounds.

The synthetic rout is outlined in Scheme 1. Commercially available cinnamic acid was used as a starting material to synthesize compounds **1**–**28**. Cinnamic acid was treated with $SOCl_2$ to provide cinnamoyl chloride. The esters (**1**–**23**), thioester (**24**) or amides (**25**–**28**) were obtained by reaction of cinnamoyl chloride with the appropriate alcohols, phenols, ethyl mercaptan, ammonia or amines in 85–94% yield. Ketone **29** was synthesized by aldol condensation reaction of benzaldehyde with 2-pentanone in 61% yield. Commercially available cinnamyl alcohol was treated with $SOCl_2$ to yield cinnamyl chloride, and followed by treatment with sodium ethoxide to yield ether **30**. Compounds **32**–**45** were obtained by Wittig reaction of ethyl triphenylphosphanylideneacetate [$(C_6H_5)_3$-P=$CHCO_2$Et] and the corresponding heterocyclic formaldehydes in ethanol in 55–92% yield.

The synthesized known compounds were confirmed by comparison of its ^{1}H, ^{13}C NMR and MS data with those reported in literature. New compound **21** was characterized by ^{1}H NMR, ^{13}C NMR and HRMS analysis. In ^{1}H NMR spectra, the esters (**1**–**23**, **32**–**45**), amides (**25**–**28**) and thioester (**24**) showed two doublet signals in the ranges of δ_H 7.5–8.0 (1H, d, J = 16.0 Hz) and 6.2–7.3 (1H, d, J = 16.0 Hz) due to H_β and H_α of the acryloyl moiety, respectively. In ^{13}C NMR spectra, the esters and amides showed signal of one carbonyl at δ_C 164.4–167.9. The carbonyl signals of thioester **24** and ketone **29** were observed at δ_C 190.0 and 200.6, respectively. Ether **30** showed one doublet signal at δ_H 6.67 (d, J = 15.9 Hz, 1H) and one double triplet signal at δ_H 6.37 (2 × t, J = 15.9, 6.0 Hz, 1H) due to two protons of the ethylene moiety, and signals of one additional methylene at δ_H 4.20 (dd, J = 6.0, 1.4 Hz, 2H) and δ_C 71.3 compared with **2**. The coupling constant values of H_α and H_β (J = *ca.* 16 Hz) confirmed *trans* configuration of the compounds.

Compounds **1**–**45** were screened for acaricidal activity *in vitro* against *P. cuniculi* according to our previously reported method.[26] Ivermectin, a clinical acaricidal drug standard, was used as a reference control. The results are listed in Table 1. All the compounds showed the activity at various degrees at 0.5 mg/mL, and of which 15 compounds (**1**–**5**, **15**, **16**, **24**, **29**, **32**–**34**, **38**, **40**, **41**) showed the mortality rates of 70–100%, equal to or higher than that of ivermectin (75.0%) ($p < 0.05$). At 0.25 mg/mL, nine compounds (**1**–**3**, **16**, **32**, **34**, **38**, **40**, **41**) showed the mortality rates of 66–100%, equal to or higher than that of ivermectin (68.0%) ($p < 0.05$). Compounds **2**, **32** and **40** gave the highest mortality rates of 98–100% ($p > 0.05$) at 0.25 mg/mL.

In order to get insight into acaricidal potency in more detail, the compounds (**1**–**3**, **16**, **32**, **34**, **38**, **40**) with the higher initial activity were further determined for median lethal concentrations (LC_{50}) and median lethal times (LT_{50}) according to the same method as described above. Ivermectin was used as a reference drug. The mortality rates caused by various test concentrations of the compounds for the same time (24 h) or various treatment times at the same concentration (4.5 mM) are shown in Figs. 2 and 3, respectively. LC_{50} and LT_{50} values of the compounds are listed in Tables 2 and 3, respectively.

It was seen from Figs. 2 and 3 that the activity of all the compounds including ivermectin increased with increase of both test concentration and treatment time. However, the various compounds showed the different change trend (or steepness) of the curves, showing that the effects of the treatment concentration or time on the activity of the various compounds are different. In most cases, the test compounds showed the higher activity at the same test mass concentration than ivermectin (Fig. 2A), but the opposite was observed at the same molar concentration (Fig. 2B). The results above were further confirmed by LC_{50} values of the various compounds (Table 2). When LC_{50} values are expressed in millimolar concentration, all the compounds (LC_{50} = 0.36–1.07 mM) were less active than ivermectin (LC_{50} = 0.28 mM). On the contrary, when mass concentration is applied, all the compounds (LC_{50} = 60–192 μg/mL) were more active than ivermectin (247.4 μg/mL). The results above are due to the molecular weight of ivermectin (MW = 875) being much higher than that of the compounds (MWs = 166–258). Although the acaricidal mechanism of cinnamic acid esters is still unknown at present, the great structural difference between ivermectin and cinnamic acid esters strongly suggests that the two class of compounds may well possess different acaricidal mechanism. In this case, the mass number of LC_{50} values should be more suitable than its molar number for comparison of the activity strength and application potential of ivermectin and the compounds. Among the compounds, **40** displayed the highest activity (LC_{50} = 0.36 mM or 60.4 μg/mL) followed by **32** (LC_{50} = 0.45 mM or 81.6 μg/mL), and their relative molar activity reach 0.78 and 0.62 that of ivermectin, respectively (Table 2).

From the change trend of the curves in Fig. 3, it was found that the sensitivity of the mites to the treatment-time change of all the compounds was much higher than ivermectin. Among them, both **38** and **40** showed the higher activity with LT_{50} values of 6.8 and 2.6 h than ivermectin (LT_{50} = 8.9 h). From the point of view of LT_{50}, the relative activity of **40** reached up to 3.4-fold that of ivermectin. It was worth mentioning that all the LT_{50} values in Table 3 were measured at the same test molar concentration (4.5 mM). Considering the fact that the molecular weight of ivermectin is much higher than the test compounds, it was speculated that most or all the compounds should have smaller or much smaller LT_{50} values than ivermectin if the same mass concentration is used for test. This inference was confirmed to some extent by the compounds in Table 3 having the higher initial activity at the same mass concentration than ivermectin (Table 1).