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Communication

Synthesis, nematicidal activity and docking study of novel chromone derivatives containing substituted pyrazole

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Graphical Abstract

Synthesis, nematicidal activity and docking study of novel chromone derivatives containing substituted pyrazole

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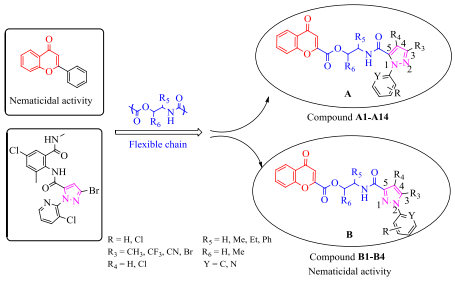

A series of chromone derivatives containing substituted pyrazole were designed and synthesized. Preliminary bioassays showed that most of the synthesized compounds exhibited good nematicidal activity *in vivo* against *Meloidogyne incognita* at 10 mg/L.

ABSTRACT

A series of chromone derivatives containing substituted pyrazole were designed and synthesized. Preliminary bioassays showed that most of the synthesized compounds exhibited good nematicidal activity *in vivo* against *Meloidogyne incognita* at 10 mg/L. Among the tested compounds, **A10** and **A11** exhibited 100% inhibition rates. In addition, the molecular docking results indicated that both compound **A10** and **A11** interacts with amino acid residue Tyr121, Trp279, Tyr70, Trp84 and Phe330 of AChE *via* hydrogen bond and *π-π* stacking. This investigation suggested that the chromone containing substituted pyrazole scaffold could be further optimized to explore novel, high-bioactivity nematicidal leads

Keywords

Chromone
Substituted pyrazole

Meloidogyne incognita
Nematicidal activity
Molecular docking

Root-knot nematodes (RKNs) are soil worms belonging to the plant-parasitic nematodes that feed on the roots of different crops such as tomato, pepper, watermelons and onions, resulting in an annual crop loss of about 100 billion USD around the world [1,2]. At present, fosthiazate and abamectin are the most commonly used nematocides in the market. Avermectin is a broad-spectrum, highly effective pesticide. It is widely used in the prevention and control of harmful insects, mites and nematodes in agriculture [3,4]. Fosthiazate is an organophosphorus nematicide, which has the advantages of non-cross resistance of most organophosphorus compounds and easy to be degraded [5-7]. Although recently some new stars of nematocide industry like fluensulfone and tioxazafen have entered the pesticide market [8-10], such as tioxazafen, a new type of broad-spectrum nematocide, mainly used for seed treatment, has a good control effect. The search for novel chemotypes of nematicides, which are safer, environmentally friendly, and nematode-specific, is still urgent [11].

Chromones are a group of naturally occurring compounds that are ubiquitous in nature, especially in plants. Both of synthetic and natural origin have been recognized to display valuable antibacterial, antifungal and antirhinoviral activity (Fig. S1 in Supporting information) [12,13]. More importantly, Lee *et al.* [14]. reported that chromone derivatives are active nematicidal compounds (Fig. S1).

It is well known that pyrazole exhibited diversity biological activities, such as antimicrobial, xanthine oxidase inhibitory activity, herbicidal [15-19]. What's more, Badische Anilin-und-Soda-Fabrik (BASF) crop science reported in 2007 that the pyrazole derivative **1**–**1** can be used as a nematicide (Fig. S2 in Supporting information) [20]. In our group's previous study, Li *et al.* [21] and Chen *et al.* [22] reported that pyrazole derivative containing a diphenylacetylene scaffold (**1-2**) and pyrazole derivative containing a 1,2,3-triazole scaffold (**1-3**) exhibited good inhibition rates against *Meloidogyne incognita* (Fig. S2). Liu *et al.* [23,24] also synthesized some pyrazole derivatives **1-4** and **1-5** (Fig. S2).

In this study, our design strategy is to construct a novel structure scaffold which was combined chromone and substituted pyrazole by flexible chiain. In 2014, Syngenta and Bayer crop science announced a series of aromatic/heterocyclic carboxamides, which have good nematocidal activity [25,26]. (Fig. S3 in Supporting information). With all this in mind, we introduced amide and alkyl ester flexible chain into chromone structure as linker to investigate the nematicidal bioactivity, and designed two series of novel chromone derivatives (Scheme 1, **A** and **B**). The chromone derivatives containing substituted pyrazole were synthesized and their biological activities *in vivo* against *M. incognita* were measured.

The general synthetic routes (Schemes S1and S2) and experimental data for synthesis of compounds **A1**-**A14** and **B1**-**B4** were listed in Supporting information. The second-stage juveniles (J2) of *M. incognita* used in all tests were cultured by Huzhou Modern Agricultural Biotechnology Innovation Center, Chinese Academy of Sciences, China. Assay of nematicidal activity can be found in Supporting information.

The *in vivo* nematocidal activity of compounds **A1**-**A14** and **B1**-**B4** against *M.incognita* are listed in Table 1. Fenamiphos and Avermectin were used as controls. As shown in Table 1, the preliminary bioassays indicated that almost all of the target compounds had exceed 90% inhibition rates against *M. incognita* at 25.0 mg/L.

Based on the data in Table 1, when we fixed chromone and substituted pyrazole two parts, and focus our attention on the effect of the linker type on the nematicidal bioactivity, it was found that R_5 and R_6 were hydrogen (compound **A1**, 100%) displayed good inhibitory activities against *M. Incognita*. However, when R_6 was hydrogen and methyl, ethyl and phenyl were introduced into R_5 as flexible chain, the nematicidal activities of compound **A2** (95.5%), **A3** (90.3%) , **A4** (91.4%) were weaker than that of **A1**, which indicated that R_5 and R_6 of flexible chain without substitutes are in benefit for increasing nematocidal activity. When chromone and flexible chain were fixed, the substituents of pyrazole were changed further. Compared with pyrazole substituted by benzene and pyridine, it was found that the bioactivity of compounds **A6** (90.3%) was higher than that of compounds **A1** (86.6%) at 10.0 mg/L. In order to identify pyridine group is benefit for increasing nematicidal activity, compound **A5**, **A7**, **A8**, and **A9** were synthesized, compounds **A5** (70.3%), **A8** (70.3%) and **A9** (80.4%) had higher nemoticidal activities than compounds **A3** (65.6%), **A2** (65.7%) and **A4** (60.0%), respectively. Meanwhile, when R_6 of flexible chain was methyl, the nematicidal activity of compound **A7** (75.9%) was also lower than that of **A6** (90.3%), which suggested further that no substitute on both R_5 and R_6 of flexible chain are benefit for increasing nematocidal activity once again. Furthermore, the influence of substitutes position of nitrogen atom of pyrazole ring were investigated, which was shown in Scheme 1. When the substitutes were R_1, compound **A1**-**A14**, correspondingly, the substitutes were R_2 in compound **B1**-**B4**. Analyzing the activity of these compounds, it was found that the nematicidal activities of **B1** (50.3%), **B2** (60.5%) , **B3** (50.4%) and **B4** (55.0%) decreased compared to that of compounds **A5 (**70.3%), **A6 (**90.3%), **A7 (**75.9%) **, A8 (**70.3%) at 10.0 mg/L. It suggest that the R_1 of pyrazole is important to the nemoticidal activity.

In some papers, it was reported that CF3 group can increase nematocidal activity [29,30]. So we tried to introduce CF3 group, and also introduce CN and Br group into pyrazole. It was found that A10 (100%), A11 (100%), A12 (96.7%) and A13 (100%) displayed excellent nematicidal activity against *M. Incognita* at 25.0 mg/L, respectively. Among the tested compounds, **A10** and **A11** still

exhibited 100 % inhibition rates at 10.0 mg/L. It demostrated the introduction of CF_3 group is really benefit for increasing nematocidal activity. Compared with the structure reported by Liu *et al.* [23,24], when pyrazole carboxamide kept the same, the highest inhibition rates of compound **1-5** (Fig. S2) and **1-4** (Fig. S2) was 59.4% at 10.0 mg/L and 100% at 40.0 mg/L, our compounds **A11** reached 100% inhibition rates at 10.0 mg/L under the same test condition. It indicated that chromone and ester flexible chain might increase the nemoticidal activities of our compounds.

As shown in Table 2, to determine the strength of activity of title compounds, the nematicidal evaluation of the compounds in 100% inhibitory activity at 10.0 mg/L, such as **A10** and **A11**, was continued at lower concentrations of 5.0 mg/L and 1.0 mg/L. However, it was found that the inhibitory activities decreased very quickly with the decreasing of the treatment concentration. The nemoticidal bioactivity of compound **A10** was 21.4% at the treatment concentration 5.0 mg/L; that of positive control tioxazafen exhibited 92.9%. The reason why the inhibitory rates decreased so quickly still needs to be investigated further. When the treatment concentration decreased to 1.0 mg/L, compound **A11** had 14.3% inhibitation rate, meanwhile, tioxazafen exhibited 28.6 % inhibition rates at 1.0 mg/L. Although the data is not very good, it still means that compound **A11** is valuable to modify further based on that of tioxazafen.

Some literatures has reported that AChE is one of the target of nematicides [31,32]. Our high active compound was also tried to investigate the mode with AChE, the binding modes between AChE (PDB:1odc) and the active compound A6 were selected as exemplified in the case of representative compound. First of all, we did the docking poses of **A6** with the AChE, and then did the compound **A10** and **A11**. Performed on Maestro 10.2 software, the most likely binding conformation was selected based on the glide score and the binding mode, which was shown in Fig. S4 (Supporting information). From the docking result, one hydrogen bond and four π-π stacking interactions were observed between the target and the compound A6. The H atom of amide group in alkyl chain of the compound A6 can form tightly interaction with the OH group of Tyr121 in the hinge domain of the target *via* a H-bond. Meanwhile, the benzene ring of the compound A6 formed the π-π stacking interaction with the Trp279 and Tyr70 residue. The pyridine ring of the compound A6 formed the π-π stacking interaction with the Phe330 and Trp84 residue. All of these interactions contributed the stability of the complex between AChE and the compound A6. It is different from the mode reported by Liu *et al.* [25,26]. Their docking results indicated that compound 1-4 (Fig. S2) interact with amino acid residue Tyr 121, Trp 279 (compound 1-5 (Fig. S2) interact with amino acid residue Tyr 121) of AChE *via* hydrogen bond. In order to verify the effect of hydrogen of CH_2CH_2 of flexible chain, we selected A5 and A8 to make a docking. The comparison of the docking A5, A6 and A8 (Fig. S4) suggests that, as the hydrogen on the flexible chain was replaced by methyl and ethyl, the number of hydrogen bonds on the flexible chain dispeared, this also explained the experimental result that when the hydrogen of CH_2CH_2 of flexible chain was replaced by methyl and ethyl groups, the nematicidal activity decreased. We also made the molecular docking of A10 and A11 with the AChE (Fig. S5 in Supporting information), both compound A10 and A11 interacts with amino acid residue Tyr121, Trp279, Tyr70, Trp84 and Phe330 of AChE *via* hydrogen bond and π-π stacking. It is the same as that of compound **A6**. The docking results also indicated that all the active compounds had similiar binding mode.

In conclusion, a novel series of chromone derivatives containing substituted pyrazole were designed and synthesized. The nematocidal activity results showed some of them possessed good activity against *M. incognita* at 10.0 mg/L. The docking results indicated that both compound **A10** and **A11** interacts with amino acid residue Tyr121, Trp279, Tyr70, Trp84 and Phe330 of AChE *via* hydrogen bond and π-π stacking. It implied that chromone containing substituted pyrazole was a potential active structure to be worth studying further.

Acknowledgments

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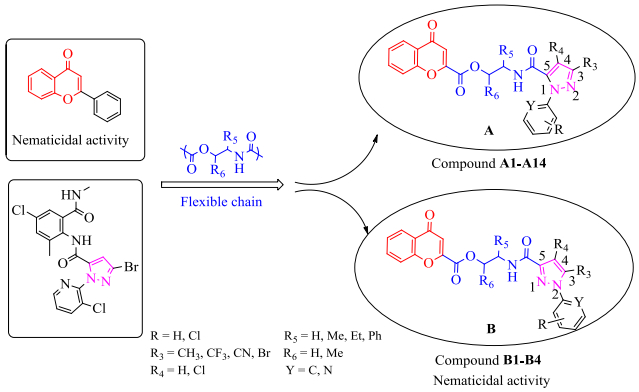
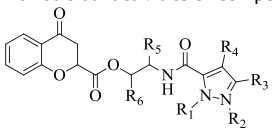


Scheme 1. The design strategy of title compounds.

Table 1
Nematicidal activities of compounds **A1**–**A14**, **B1**–**B4** against J2 of *M. incognita* in test tubes.



Compound	R1	R2	R3	R4	R5	R6	Inhibition rate against J2 of *M. Incognita* (%)[a]	
							25.0 mg/L	10.0 mg/L
A1	-phenyl	-	$-CH_3$	-Cl	-H	-H	100	86.6
A2	-phenyl	-	$-CH_3$	-Cl	$-CH_2CH_3$	-H	95.5	65.7
A3	-phenyl	-	$-CH_3$	-Cl	$-CH_3$	-H	90.3	65.6
A4	-phenyl	-	$-CH_3$	-Cl	-phenyl	-H	91.4	60.0
A5	-3-Chloropyridin-2-yl	-	$-CH_3$	-Cl	$-CH_3$	-H	100	70.3
A6	-3-Chloropyridin-2-yl	-	$-CH_3$	-Cl	-H	-H	100	90.3
A7	-3-Chloropyridin-2-yl	-	$-CH_3$	-Cl	-H	$-CH_3$	90.8	75.9
A8	-3-Chloropyridin-2-yl	-	$-CH_3$	-Cl	$-CH_2CH_3$	-H	100	70.3
A9	-3-Chloropyridin-2-yl	-	$-CH_3$	-Cl	-phenyl	-H	100	80.4
A10	-phenyl	-	$-CF_3$	-H	-H	-H	100	100
A11	-3-Chloropyridin-2-yl	-	$-CF_3$	-H	-H	-H	100	100
A12	-3-Chloropyridin-2-yl	-	-CN	-H	-H	-H	96.7	72.8
A13	-phenyl	-	-CN	-H	-H	-H	100	95.3
A14	-3-Chloropyridin-2-yl	-	-Br	-H	-H	-H	pt	60.7
B1	-	-3-Chloropyridin-2-yl	$-CH_3$	-Cl	$-CH_3$	-H	100	50.3

B2	-	-3-Chloropyridin-2-yl	$-CH_3$	-Cl	-H	-H	100	60.5
B3	-	-3-Chloropyridin-2-yl	$-CH_3$	-Cl	-H	$-CH_3$	90.8	50.4
B4	-	-3-Chloropyridin-2-yl	$-CH_3$	-Cl	$-CH_2CH_3$	-H	91.4	55.0
AVM							100	100
Fenamiphos							100	100
Tioxazafen							100	92.9

-: no substitute.AVM: Avermectin.
pt: phytotoxic.
[a] Average of three experiments.

Table 2
Nematicidal activities of compounds **A10** and **A11** against J2 of *M. incognita* in test tubes.

Compound	Inhibition rate (%)	
	5.0 mg/L	1.0 mg/L
A10	21.4	0
A11	-	14.3
AVM	100	100
Fenamiphos	100	100
Tioxazafen	92.9	28.6

-: no data because of abnormal experiment.