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Synthesis, nematicidal evaluation, and SAR study of benzofuran derivatives containing 2-carbonyl thiophene

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ABSTRACT

Plant-parasitic nematodes are major threat for crop protection. The lack of nematicides with new mode of action and increasing resistance raises the need for novel nematicides. In order to seek new nematicidal lead, originating from the structure of chalcone, a series of fused ring compounds was obtained by ring closure design strategy. These compounds were modified further. The nematicidal activity against *M. incognita* of synthesized compounds was evaluated. The bioassay showed that compound **3** and some of its derivatives such as compounds **18**, **19**, **21**, **22**, **23**, **24** and **26** exhibited excellent nematicidal activity. Among them, compound **23** exhibited significant bioactivity. The $LC_{50/72\,h}$ value reached 3.20 mg/L *in vitro* and the inhibition rate was 100.00% at 40 mg/L in the matrix. The structure-activity relationship of synthesized compounds was discussed in details. The influence of compound **23** on egg hatching, motility, and feeding behavior of *C. elegans* was also evaluated.

Plant-parasitic nematodes (PPNs) are one of the major threats to agriculture and account for 14% of the global crop yield losses which is nearly 173 billion dollars annually [1,2]. Root-knot nematode (*Meloidogyne* spp.) is one of the most destructive groups of PPNs [3]. If the plants were infected by root-knot nematodes, the characteristic such as stunted growth, wilting, leaf discoloration, and deformation of the roots would appear [4]. In addition, root-knot nematodes can interact with other pathogens and develop disease complexes, such as *Fusarium* wilt, *Rhizoctonia solani,* and *Thielaviopsis basicola*, which caused a greater loss of plants [4,5].

Synthetic nematicides play a prominent role in nematode management for their high efficiency and low cost [6]. For decades, PPNs have been mainly controlled by organophosphates, carbamates, and soil fumigants [7]. However, most of them were gradually banned or restricted due to their environmental risks and high toxicity [3,8]. So far, only a few nematicides have developed, including Fosthiazate, Fluensulfone, Fluopyram, Tioxazafen, Cyclobutrifluram, and Fluazaindolizine (Fig. 1) [9–12]. Therefore, the research and development of new nematicidal molecules are essential for the management of PPNs.

At present, the mode of actions of six new commercialized nematicides except Fosthiazate and Fluopyram are still unknown. The absence of target information makes the lead discovery based on target difficult. Thus, bioisosterism and scaffold hopping might be an applicable strategy for the exploration of nematicidal lead [13]. Ring closure is a common scaffold hopping strategy for discovering novel structures in agrochemical design [14]. The physicochemical properties and the binding free energy of the molecule would be changed through ring closure which further affects the activity and stability [15,16].

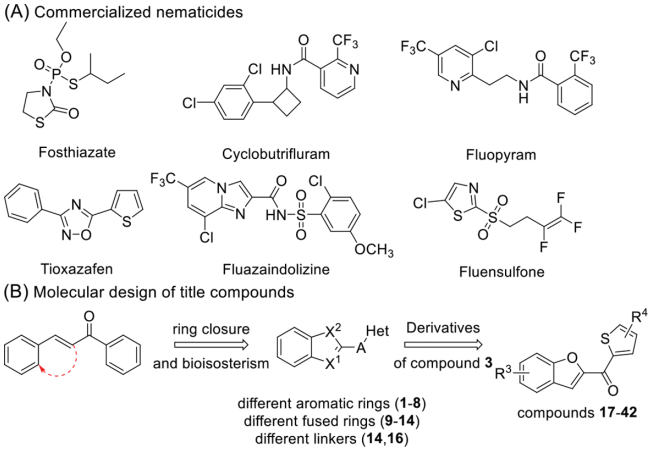

Fig. 1. Chemical structures of commercialized nematicides (A) and molecular design of title compounds (B).

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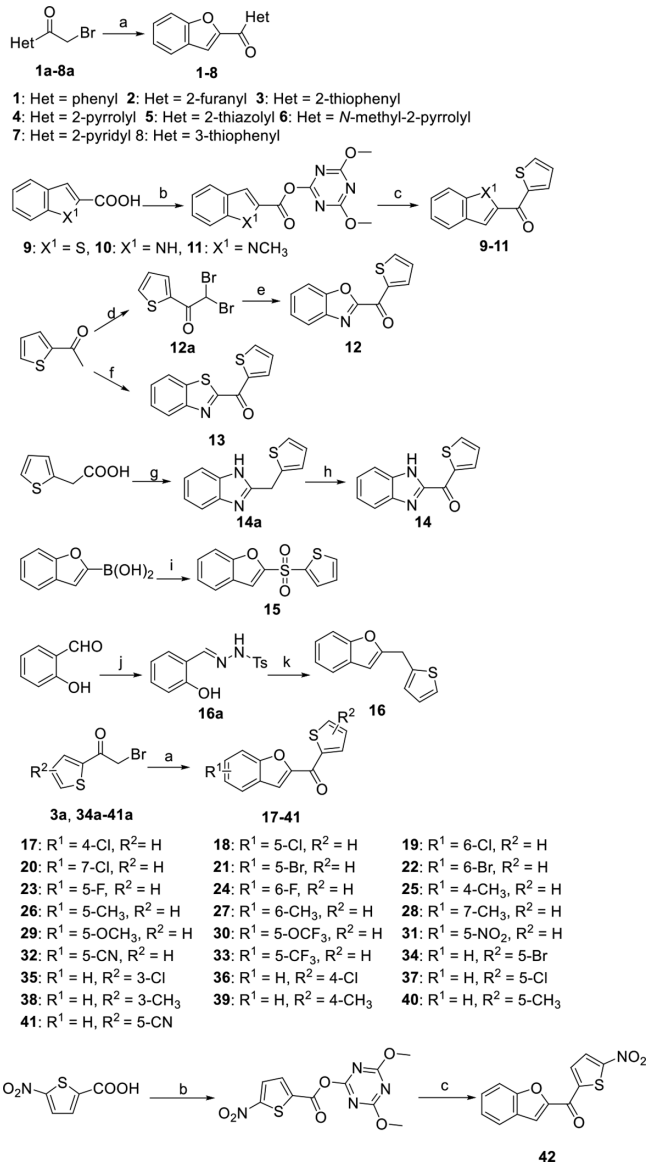


Scheme 1. Synthesis of the target compounds **1–42**. Reagents and conditions: (a) corresponding salicylaldehyde, MeCN, 81 °C; (b) CDMT, NMM, tolune, 1 h; (c) corresponding boric acid, $Pd(PPh_3)Cl_2$, K_3PO_4, tolune, 110 °C; (d) $CuBr_2$, AcOH, MeCN, 81 °C; (e) 2-aminophenol, diethylamine, DMF, 90 °C; (f) benzothiazole, I_2, KOH, DMSO, H_2O, 100 °C; (g) *o*-phenylenediamine, HCl, H_2O, reflux; (h) Cs_2CO_3, DMF, 90 °C; (i) 2-thiophenesulfonyl chloride, [(phen)Cu^{I}Br], K_2CO_3, CH_2Cl_2, H_2O, r.t.; (j) $TsNHNH_2$, MeOH, 60 °C, (k) 2-ethynylthiophene, CuBr, Cs_2CO_3, MeCN, 81 °C.

Chalcone is a scaffold with various pesticidal activity including insecticidal, fungicidal, herbicidal, and nematicidal activity [17–20]. However, chalcone is reported to have poor photostability and could be converted to various by-products under UV light [16]. It is unacceptable as a pesticide. To overcome this disadvantage, based on the structure of chalcone, we designed a series of fused ring derivatives containing 2-carbonyl heterocycles *via* ring closure strategy (Fig. 1). Then further optimization was carried out based on bioassay results. Forty-two compounds containing fused rings were synthesized. *In vitro* and *in vivo* nematicidal activity was evaluated and the structure-activity relationship (SAR) was investigated. In addition, in order to explore the possible mode of action, the influence on the egg hatching rate, motility, and feeding behavior of *C. elegans* was also studied.

The synthesis route of title compounds was shown in Scheme 1. The synthetic procedures of intermediates and title compounds were presented in the supporting information. Compounds **1–8** and **17–41** were synthesized by Rap-Stoermer reaction [21,22]. Firstly, intermediates **1a-3a**, **5a**, **6a**, **8a** and **34a-41a** were synthesized from corresponding 2-acetylaromatic heterocycles and $CuBr_2$ in ethyl acetate or THF. Meanwhile, intermediate **4a** was synthesized from pyrrole and 2-bromoacetyl bromide by Friedel-Crafts acylation with a low yield. Intermediate **7a** was purchased. Then, the corresponding intermediates reacted with different substituted salicylaldehyde in acetonitrile and got compounds **1–8** and **17–41**. The yields of most of the compounds were over 70% except those of compounds **6**, **31** and **32** which were 55%, 45% and 51%, respectively. Coincidentally, a small quantity of **12a**, a key intermediate of compound **12**, was detected by GS-MS during the synthesis of intermediate **3a**. We optimized the synthetic procedure of intermediate **3a** and applied it to the synthesis of intermediate **12a**. When 2.4 equiv. of $CuBr_2$ and 1 equiv. of acetic acid were added into brominate in acetonitrile, the yield of intermediate **12a** raised to 65%.

Compounds **9–11** and **42** were synthesized by specific Suzuki coupling [23]. The corresponding acid reacted with 2–chloro-4,6-dimethoxy-1,3,5-triazine (CDMT) and then reacted with 2-thiopheneboronic acid or benzofuran-2-boronic acid to obtain product in one pot. The yield of compounds **9–11** was more than 50%. However, that of compound **42** was just 35%. This might be due to the electron-withdrawing effect of the nitro group on the thiophene ring. Compound **12** was obtained by the cyclization of 2-aminophenol with intermediated **12a** which was prepared by the above method and the yield of compound **12** was 42% [24]. Compound **13** was synthesized from benzothiazole and 2-acetylthiophene in a one-step reaction with a yield of 65% [25]. Compound **14** was oxidized through intermediate **14a** which was obtained by the synthetic method of dibazol with a yield of 35% [26,27]. Compound **15** was prepared from benzofuran-2-boronic acid and 2-thiophenesulfonyl chloride with a copper catalyst and the yield of compound **15** was 22% [28]. Compound **16** was prepared in two steps. The reaction of salicylaldehyde with *p*-toluenesulfonyl hydrazide gave the corresponding hydrazone, intermediate **16a**. Then it was cyclized with 2-ethynylthiophene to achieve compound **16**. The yield of compound **16** was 55% [29].

The *in vitro* nematicidal activity of compounds **1–16** against *M. incognita* was shown in Table 1. The bioassay method of nematicidal activity was introduced in supporting information. To explore the SAR, different aromatic rings, fused rings, and linkers were introduced into the structure and sixteen compounds were synthesized and tested. Compounds **1–8** were firstly synthesized to explore the influence of different aromatic rings on nematicidal activity. Among these compounds, only compounds **2** and **3**, in which aromatic rings were thiophene and furan, showed *in vitro* nematicidal activity against *M. incognita* after 72 h exposure, and the LC_{50}/72 h (LC_{50} value at 72 h) of compounds **2** and **3** were 19.46 and 13.42 mg/L, respectively. The nematicidal activity of compound **3** bearing thiophene was superior to that of compound **2** bearing furan. Meanwhile, the influence of substitution positions of aromatic rings was confirmed by the obvious activity difference between compounds **3** and **8**, that is, the nematicidal activity of 2-substituted analogues was better than that of 3-substituted analogues. Then SAR of different fused ring replacements was explored based on the bioactivity results of compounds **3** and **9–14**. Compound **3** with benzofuran, compound **9** with benzothiophene, and compound **13** with benzothiazole exhibited better *in vitro* nematicidal activity against *M. incognita* after 72 h exposure and the mortality was 85.68%, 82.73%, and 70.38% at 40 mg/L, respectively. The $LC_{50/72\,h}$ of compounds **3**, **9** and **13** were 13.42 mg/L, 18.04 mg/L, and 27.47 mg/L, respectively. Compound **3** possessed the best nematicidal activity among these three compounds. Finally, the linker was optimized by replacing the carbonyl group with the sulfonyl

Table 1
In vitro nematicidal activity of compounds **1**–**16** against *M. incognita* after 72 h.

Compd.	Corrected mortality[a] (%)	$LC_{50/72\,h}$ (mg/L)	Toxic regression equation	r^2
1	18.12 ± 0.33	n.t.[b]		
2	79.02 ± 0.68	19.46	$y = 3.88x - 5.00$	0.97
3	85.68 ± 1.92	13.42	$y = 3.41x - 3.85$	0.96
4	4.68 ± 0.82	n.t.		
5	3.49 ± 0.23	n.t.		
6	1.45 ± 0.85	n.t.		
7	2.44 ± 0.71	n.t.		
8	4.62 ± 1.90	n.t.		
9	80.87 ± 0.68	18.04	$y = 4.43x - 5.57$	0.97
10	31.24 ± 2.52	n.t.		
11	2.80 ± 1.88	n.t.		
12	8.27 ± 2.76	n.t.		
13	77.07 ± 0.99	27.47	$y = 3.44x - 4.95$	0.95
14	5.09 ± 0.88	n.t.		
15	1.98 ± 0.16	n.t.		
16	2.55 ± 0.48	n.t.		
Fosthiazate	100.00	1.40	$y = 6.79x - 1.00$	0.95

[a] The concentration of the test compound is 40 mg/L. Fosthiazate was used for a comparison of activity. Each value is the average ± SD of three independent biological replicates.
[b] n.t.: not test.

group (compound **15**) or the methylene group (compound **16**). But neither of these two compounds exhibited *in vitro* nematicidal activity against *M. incognita* after 72 h exposure. This suggests that the carbonyl group was important for the nematicidal activity. Based on the above results, compound **3**, whose structure was benzofuran-2-yl(thiophen-2-yl)methanone, was confirmed as the most active scaffold among the synthesized compounds and then the derivatives of compound **3** (compounds **17–42**) were synthesized and their nematicidal activity was tested subsequently.

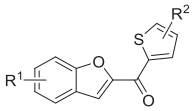
The *in vitro* nematicidal activity of compounds **3** and **17–42** against *M. incognita* was shown in Table 2. When the substituents were introduced into benzofuran ring of compound **3**, compounds **18** (R^1 = 5-Cl), **19** (R^1 = 6-Cl), **21** (R^1 = 5-Br), **22** (R^1 = 6-Br), **23** (R^1 = 5-F), **24** (R^1 = 6-F), **26** (R^1 = 5-CH_3), **27** (R^1 = 6-CH_3) and **30** (R^1 = 5-OCF_3) exhibited good nematicidal activity against *M. incognita* after 72 h exposure. It was obvious that 5- and 6-substituted compounds demonstrated better nematicidal activity than others after 72 h exposure. Furthermore, the nematicidal activity difference at these two substitution positions was confirmed. The *in vitro* nematicidal activity of 5-substituted compounds was better than those of 6-substituted compounds. Compound **26** (5-CH_3, 96.00%) was superior to that of compound **27** (6-CH_3, 59.03%). Among 5- and 6-substituted compounds, the mortality of compounds with halogen substituents reached 100.00% after 72 h exposure. Moreover, both compounds with weak electron-withdrawing or electron-donating substituents [(compounds **26** (R^1 = 5-CH_3), **27** (R^1 = 6-CH_3), and **30** (R^1 = 5-OCF_3)] possessed good nematicidal activity against *M. incognita* at 72 h, the mortality of them were 96.00%, 59.03% and 62.41% at 40 mg/L, respectively. But those of compounds with strong electron-withdrawing or electron-donating substituents [(compounds **31** (R^1 = 5-NO_2), **32** (R^1 = 5-CN), **33** (R^1 = 5-CF_3) and **29** (R^1 = 5–OCH_3)] lost their activity at 72 h. In order to further compare the activity of these active compounds, $LC_{50/72\,h}$ of these active compounds were tested. Among these nine compounds, the activity of compounds **18**, **23** and **24** was better than others and the $LC_{50/72\,h}$ values were 4.60, 3.20 and 3.80 mg/L, respectively, which was similar to that of Fosthiazate. It was also found when the substituents of the compounds located at position 5, the nematicidal order could be briefly summarized as follows: -F > -Cl > -Br > -H > -CH_3 > -OCF_3 >> others. It indicated that only the halogen substituents on the benzofuran ring would enhance the nematicidal activity compared with compound **3**. Unexpectedly, the substituents on thiophene decreased *in vitro* nematicidal activity of compound **3**. Among compounds **34–42**, none exhibited *in vitro* nematicidal activity after 72 h exposure. Compound **23** (R^1 = 5-F) exhibited the best *in vitro* nematicidal among all forty-two compounds.

The *in vivo* nematicidal activity of compounds **3**, **18**, **19**, **21**, **22**, **23**, **24** and **26** were subsequently tested and presented in Table 2. Most of these compounds exhibited obvious control effects against *M. incognita* at 40 mg/L in the matrix. The inhibition rates of compounds **18**, **19**, **21**, **23** and **24** were greater than 65%, which were 73.42%, 68.25%, 69.80%, 100.00%, 88.22%, respectively. The SAR rule of the *in vivo* nematicidal activity was the same as that of *in vitro*, the activity of 5-substituted compounds was superior to that of 6-substituted. For example, the inhibition rate of compound **23** (R^1 = 5-F, 100.00%) was higher than that of compound **24** (R^1 = 6-F, 88.22%). At the same time, the bioactivity of compound **23** bearing fluoro group was better than compounds bearing other groups at the same substitution position.

Generally, it is hard to directly observe the behavior of *M. incognita* in lab conditions because they spend most of their time in soil and roots [30]. *C. elegans* is a typically model organism in the research of PPNs and nematicide [10,31]. In order to explore the influence of title compounds on the nematode, the effect of compound **23** with the best nematicidal activity on egg hatching, motility, and feeding behavior was investigated, which might be relative to the process of infecting the root of plants. The detail of the test was described in the supporting information. Fluensulfone was selected as a positive control, which has been reported to inhibit egg hatching, motility, and feeding behavior of *C. elegans* [32].

The results in Table S1 (Supporting information) suggested that compound **23** inhibited significantly egg hatching of *C. elegans* after 24 h and 48 h exposure. The egg hatching rate did not increase with the extension of time. When the concentration of compound **23** was 10 mg/L, only 5.83% of eggs were hatched at 48 h. Meanwhile, egg hatching rates treated with Fluensulfone at the same concentration and pure M9 buffer (with DMSO, negative control) were 87.82% and 91.52% at 48 h, respectively. Evidently, eggs treated with compound **23** have a lower egg hatching rate than that of Fluensulfone. When the concentration of compound **23** was higher than 2.5 mg/L, the inhibition rate of egg hatching was more than 70%. The egg hatching inhibition was still observed even when the concentration was reduced to 0.625 mg/L. It is distinct that the higher concentration of compound **23** increased, the lower the egg hatching rate was. We guessed that compound **23** may act on nematode chitinase due to its high egg hatching inhibition rate.

Table 2
In vitro and *in vivo* nematicidal activity of compounds **17**–**42** against *M. incognita* after 72 h.



Compd.	R^1	R^2	Corrected mortality[a] (%)	$LC_{50/72\ h}$ (mg/L)	Toxicity regression equations	r^2	Inhibition rate (%) at 40 mg/L *in vivo* (in matrix)
3	H	H	85.68 ± 1.92	13.42	$y = 3.41x$ - 3.85	0.96	53.09 ± 6.00
17	4-Cl	H	8.52 ± 1.70	n.t.[b]			
18	5-Cl	H	100.00	4.60	$y = 3.44x$ - 2.28	0.97	73.42 ± 3.66
19	6-Cl	H	100.00	8.79	$y = 5.08x$ - 4.78	0.98	68.25 ± 5.91
20	7-Cl	H	6.94 ± 1.54	n.t.			
21	5-Br	H	100.00	7.33	$y = 4.62x$ - 4.00	0.99	69.80 ± 4.89
22	6-Br	H	100.00	14.22	$y = 3.14x$ - 3.62	0.94	8.97 ± 4.34
23	5-F	H	100.00	3.20	$y = 3.78x$ - 1.91	0.94	100.00
24	6-F	H	100.00	3.80	$y = 3.30x$ - 1.91	0.98	88.22 ± 5.91
25	4-CH_3	H	2.91 ± 0.20	n.t.			
26	5-CH_3	H	96.00 ± 3.89	14.20	$y = 4.24x$ - 4.89	0.99	41.93 ± 3.82
27	6-CH_3	H	59.03 ± 1.80	35.83	$y = 2.95x$ - 4.58	0.95	n.t.
28	7-CH_3	H	5.22 ± 1.07				
29	5–OCH_3	H	21.68 ± 1.19	n.t.			
30	5-OCF_3	H	67.27 ± 1.29	26.05	$y = 2.53x$ - 3.58	0.96	n.t.
31	5-NO_2	H	3.97 ± 0.11	n.t.			
32	5-CN	H	2.24 ± 0.58	n.t.			
33	5-CF_3	H	2.39 ± 0.74	n.t.			
34	H	5-Br	1.76 ± 0.53	n.t.			
35	H	3-Cl	5.45 ± 1.16	n.t.			
36	H	4-Cl	1.62 ± 0.55	n.t.			
37	H	5-Cl	2.63 ± 0.35	n.t.			
38	H	3-CH_3	33.08 ± 0.30	n.t.			
39	H	4-CH_3	2.65 ± 0.64	n.t.			
40	H	5-CH_3	4.61 ± 0.35	n.t.			
41	H	5-CN	1.87 ± 0.11	n.t.			
42	H	5-NO_2	2.23 ± 0.49	n.t.			
Fosthiazate			100.00	1.40	$y = 6.79x$ - 1.00	0.95	100.00
Negative control[c]							0.00[d]

[a] The concentration of the test compound is 40 mg/L. Fosthiazate was used for a comparison of activity. Each value is the average ± SD of three independent biological replicates.
[b] n.t.: not test.
[c] Negative control for *in vivo* test.
[d] Negative control was treated with distilled water containing acetone and Triton x-100 only.

In order to further evaluate the influence of compound **23** on the nematodes, we also counted thrashes and pumping frequency of *C. elegans* to investigate its effect on motility and feeding behavior. As shown in Table S2 (Supporting information), the thrashes of *C. elegans* decreased with the increase of the concentrations of compound **23** and Fluensulfone. The thrashes were obviously inhibited when the concentration of compound **23** was 40 mg/L, which was nearly 42 thrashes during 30 s with an approximately 34% of inhibition rate. Meanwhile, Fluensuflone has a similar inhibition effect on thrashes, which was nearly 39 thrashes during 30 s. Furthermore, the effect of compound **23** on the feeding behavior of *C. elegans* was also investigated. However, compound **23** slightly inhibited the pumping frequency of *C. elegans*, the effect was very weak compared with that of Fluensuflone, which was shown in Table S3 (Supporting information).

Although some explorations have been performed, the mode of action of compound **23** is unknown. Based on the activity *in vitro*, it suggested that compound **23** could act directly on nematodes themselves, but the interaction with target enzyme is weak compared to that of Fosthiazate. In addition, due to the high egg hatching inhibition rate of compound **23**, we guessed that nematode chitinase may be a possible target of our compounds. There are more works to do to confirm our speculation.

In conclusion, a series of novel nematicidal compounds with benzofuran-2-yl(thiophen-2-yl)methanone derivatives were rationally designed. Some of the synthesized compounds displayed excellent nematicidal activity which was similar to the commercialized nematicide Fosthiazate. Among these compounds, compound **23** showed the best activity against *M. incognita* both *in vitro* and *in vivo*, meanwhile, it also exhibited inhibiting influence on egg hatching, and motility of *C. elegans* which implied that compound **23** might have a similar influence on *M. incognita* and further inhibited the behavior of *M. incognita* in the process of infecting the root of plants. These results suggested that the structure of benzofuran containing 2-carbonyl thiophene could be considered as a potential nematicidal lead for further structural optimization.

Declaration of competing interest

The authors declare that they have no competing interests.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.cclet.2022.107800.

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