Synthesis and Nematicidal Activities of 1,2,3-Benzotriazin-4-one Derivatives against *Meloidogyne incognita*

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ABSTRACT: A series of novel 1,2,3-benzotriazin-4-one derivatives were synthesized by the reaction of 3-bromoalkyl-1,2,3-benzotriazin-4-ones with potassium salt of 2-cyanoimino-4-oxothiazolidine in the presence of potassium iodide. Nematicidal assays *in vivo* showed that some of them exhibited good control efficacy against the cucumber root-knot nematode disease caused by *Meloidogyne incognita*, up to 100% at the concentration of 10.0 mg L^{-1}, which indicated that 1,2,3-benzotriazin-4-one derivatives might be potential for novel promising nematicides. The nematicidal activity was influenced by the combination of substituent type, substituted position and linker length in the molecule. The inhibition rate data at the concentrations of 5.0 and 1.0 mg L^{-1} for the compounds with high inhibitory activities were also provided. When tested *in vitro*, none of them showed direct inhibition against *M. incognita*. The investigation of significant difference between *in vivo* and *in vitro* data is in progress.

KEYWORDS: 1,2,3-benzotriazin-4-one, 2-cyanoiminothiazolidin-4-one, nematicide, *Meloidogyne incognita*, *in vivo*

INTRODUCTION

Plant-parasitic nematodes cause an annual loss of $157 billion to world crops.[1] Root-knot nematodes (*Meloidogyne* spp.) are one of the most severe plant-damaging parasitic nematodes, attacking over 3000 plant species throughout the world.[2,3] Four major species among them, namely *M. incognita*, *M. javanica*, *M. arenaria*, and *M. hapla*, are responsible for approximately 95% of all damages caused by RKN.[4] *M. incognita*, as one of the most important plant-parasitic species,[5] cause the formation of large galls or 'knots' in roots of infected plants, and sequential physiological plant disorders.[6]

For decades, chemical nematicides have played a prominent role in the management of nematodes,[7] which are efficacious, easy to apply and rapid-onset.[8] Dibromochloropropane (DBCP), known as an effective organochlorine nematicide, has been banned since 1979 because of its mutagenicity, carcinogenicity and reproductive effects on human.[9] Methyl bromide has been used widely as a fumigant nematicide before it is restricted owing to its detrimental effects on ozonosphere.[10] Albeit with a long and controversial history in terms of environmental contamination and adverse impacts on human health, the development of chemical nematicides have never stopped. To date, a few commercial organophosphorous and carbamate nematicides are mainly applied for the control of root-knot nematodes, such as ethoprophos, fosthiazate, and oxamyl,[11] and their repeated application has given rise to the resistance development in the target pathogens.[12] The macrocyclic

lactone avermectin is commonly utilized as seed treatment nematicide because of its low water solubility and light unstability.[13,14] Recently, the newly developed trifluorobutenesulfanyl nematicide fluensulfone has been released to the market, which exhibits high nematicidal activity against root-knot nematodes and a different mode of action compared to conventional nematicides (Figure 1, compound **1**).[15-17] Tioxazafen, a new class of 1,2,4-oxadiazole nematicide with excellent broad-spectrum activity, is still undergoing worldwide development (Figure 1, compound **2**).[18] And some other nitrogen-containing heterocyclic compounds having certain nematicidal potential are also reported (Figure 1, compounds **3**–**7**).[19-23] Besides, botanical nematicides based on plant secondary metabolites have gained much scientific interest throughout the world. The majority of them are natural product extracts or their simple derivatives,[5,6,24,25] and none has proven to be very effective and used in the field. With the growing awareness of the adverse impact on environment and human health,[5,6,17] organophosphorous and carbamate nematicides will not adapt to the sustainable development of modern agriculture any more, and the number of nematicidal agents available to effectively control nematodes will become more limited. Therefore, the search for novel chemotypes of nematicides, which are safer, environmentally friendly, and nematode-specific, has become increasingly urgent.[26]

Triazinone, as a heterocyclic skeleton associated with a wide range of pesticide activities (Figure 2, compounds **8**–**12**),[27-31] has attracted much attention. Metribuzin

is well-known as a widely used triazinone herbicide (Figure 2, compound **8**).[27] Compounds **9** and **10** exhibited plant-growth regulatory and insecticidal activities, respectively.[28,29] The 1,2,3-benzotriazin-4-one derivative **11** was first reported to have nematicidal activity against *Anguillula* nematodes in 1960,[30] and compound **12** had certain nematicidal activity against *Caenorhabditis elegans*.[31] However, little work has been done on nematicidal 1,2,3-benzotriazin-4-one derivatives against plant-parasitic nematodes.

During the design of 1,2,3-benzotriazin-4-one derivatives, we found that thiazolidin-4-one compounds exhibited good inhibitory activities against *M. incognita*[20] and the neonicotinoid derivative **3** had significant nematicidal activity against *M. incognita* at the concentration of 20.0 mg L^{-1}.[19] Hence, we attempted to introduce 2-cyanoiminothiazolidin-4-one moieties into the 1,2,3-benzotriazin-4-one structure, while the active fragment of compound **3** was also considered, aiming to obtain more active compounds against *M. incognita* (Figure 3).

In the present study, we reported for the first time 1,2,3-benzotriazin-4-one derivatives containing 2-cyanoiminothiazolidin-4-one moieties and their *in vivo* and *in vitro* biological activities against *M. incognita*. We also discussed the relationship between structure and nematicidal activity preliminarily and tried to illuminate the mechanism of our compounds against nematodes.

MATERIALS AND METHODS

Chemicals. Anthranilic acids (**I-1**–**13**) and triphosgene were purchased from

Shanghai Darui Fine Chemicals Co., Ltd. Ammonium carbonate, sodium nitrite, hydrochloric acid, potassium hydroxide, potassium carbonate, anhydrous sodium sulfate and all solvents were purchased from Shanghai Lingfeng Chemical Reagent Co., Ltd. *N*-cyanodithiocarbonimidate, 1,2-dibromoethane, 1,3-dibromopropane, 1,4-dibromobutane, 1,5-dibromopentane, 1,6-dibromohexane and potassium iodide were purchased from Shanghai Aladdin Chemistry Co., Ltd. All reagents and solvents were of reagent grade without further purification.

Instrumental Analysis. Analytical thin-layer chromatography (TLC) was performed on precoated glass plates (silica gel 60 F_{254}), visualizing the spots by a UV lamp (254 nm). All melting points were determined on a Büchi Melting Point B540 apparatus (Büchi Labortechnik AG, Flawil, Switzerland) and are uncorrected. ^{1}H, ^{13}C, and ^{19}F NMR spectra were recorded in DMSO-d_6 on a Bruker AM-400 (400 MHz) spectrometer at ambient temperature. Chemical shifts are reported in δ (parts per million) values with tetramethylsilane (TMS) as the internal standard. Coupling constants (J) are reported in hertz. High-resolution mass spectra (HR-MS) were recorded under electro-spray ionization condition on a Waters Micromass LC-TOF spectrometer.

General Procedures. *Synthesis of Isatoic Anhydrides*[32] ***(II-1–13)***. A mixture of anthranilic acid (**I-1**–**13**, 40 mmol) and tetrahydrofuran (THF, 100 mL) was stirred at -10 ºC for 30 min. Then a solution of triphosgene (BTC, 40 mmol) in THF (20 mL) was added dropwise to the above mixture. After that, the mixture was stirred for

1 h at -10 ºC – -5 ºC, followed by 18 h at room temperature. The solvent was removed under reduced pressure, and anhydrous ether (150 mL) was added to the obtained residue with vigorous stirring. The precipitate was collected by filtration, washed with anhydrous ether, and dried to afford **II-1**–**13** in yields of 85–95%.

Synthesis of Anthranilamides[33] ***(III-1–13)***. A suspension of isatoic anhydride (**II-1**–**13**, 35 mmol), ammonium carbonate (140 mmol) and 1,4-dioxane (150 mL) was heated at 60 ºC. After stirring for 5–8 h, the reaction mixture was cooled to room temperature, evaporated under reduced pressure, and then water (200 mL) was added to the residue, which was extracted with EtOAc (80 mL × 3). The organic layer was washed with brine (80 mL), dried over anhydrous Na_2SO_4, and concentrated to give **III-1**–**13** in yields of above 73–94%.

Synthesis of 1,2,3-Benzotriazin-4-ones[34] ***(IV-1–13)***. A solution of sodium nitrite (4.14 g, 60 mmol) in 0.5 N HCl (240 mL) was stirred at 0 ºC for 20 min. Then anthranilamide (**III-1**–**13**, 30 mmol) dissolved in *N*, *N*-dimethylformamide (DMF, 15 mL) was added dropwise to the above solution for 40 min. After another 1 h of stirring at 0 ºC, 30% aqueous ammonia was added slowly to adjust the pH to 10.0. The reaction mixture was allowed to stir vigorously for 15 min, and then reacidified to pH 2.0. After stirring for 30 min, the precipitated product was filtered off with suction, washed with water (200 mL) and dried to afford **IV-1**–**13** in yields of above 84–92%.

Synthesis of Potassium Salt of 2-Cyanoimino-4-oxothiazolidine.[35] A stirring

mixture of dimethyl *N*-cyanodithiocarbonimidate (11.68 g, 80 mmol), ammonium carbonate (4.22 g, 44 mmol) and EtOH (120 mL) was heated at 60 ºC for 3 h. To this, methyl thioglycolate (7.15 mL, 80 mmol) and 50% KOH (aq, 8.96 g, 80 mmol) were added separately in one portion. The reaction mixture was heated to reflux for 5 h, and then cooled to room temperature. The precipitate was collected by filtration, washed with cold EtOH, and dried to give the desirable potassium salt as a light yellow solid, yield 73%.

Synthesis of 3-Bromoalkyl-1,2,3-benzotriazin-4-ones[36] ***(V-1–23)***. A stirring suspension of 1,2,3-benzotriazin-4-one (**IV-1**–**13**, 6 mmol), dibromoalkane (30 mmol), potassium carbonate (1.66 g, 12 mmol) and acetone (50 mL) was refluxed for 5–8 h, and then cooled to room temperature. The reaction mixture was evaporated under reduced pressure, and water (100 mL) was added to the residue, which was extracted with CH_2Cl_2 (50 mL × 2). The organic layer was dried over anhydrous Na_2SO_4, concentrated and purified with flash chromatography on silica gel, eluting with petroleum ether (60–90 ºC)/EtOAc to afford **V-1**–**23** in yields of 46–62%.

General Synthetic Procedure for Title Compounds ***(VI-1–23)***. To a mixture of **V** (2 mmol), potassium iodide (0.332 g, 2 mmol) and DMF (10 mL), potassium salt of 2-cyanoimino-4-oxothiazolidine (0.358 g, 2 mmol) was added, and then the mixture was stirred at 100 ºC. The reaction process was monitored by TLC. After the complete consumption of **V**, the reaction was cooled to room temperature. The

142 solvent was removed under reduced pressure, and water (50 mL) was added to the
143 residue, which was extracted with CH_2Cl_2 (30 mL × 2). The organic layer was dried
144 over anhydrous Na_2SO_4, concentrated and purified with flash chromatography on
145 silica gel, eluting with petroleum ether (60–90 ºC)/EtOAc to afford **VI-1**–**23**. The
146 data of **VI-1**–**10** are shown as follows, whereas data of **VI-11**–**23** are deposited in
147 Supporting Information.

148 *N-(4-Oxo-3-(3-(4-oxobenzo[d][1,2,3]triazin-3(4H)-yl)propyl)thiazolidin-2-yliden*
149 *e)cyanamide (**VI-1**)*: yield, 41%; mp 156.9–157.6 ºC; ^{1}H NMR (400 MHz,
150 DMSO-d_6) δ 8.26 (d, J = 7.6 Hz, 1H), 8.21 (d, J = 8.0 Hz, 1H), 8.10 (t, J = 7.6 Hz,
151 1H), 7.94 (t, J = 7.6 Hz, 1H), 4.41 (t, J = 7.2 Hz, 2H), 4.30 (s, 2H), 3.74 (t, J = 7.2
152 Hz, 2H), 2.21–2.09 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 179.6, 173.0, 155.3,
153 144.1, 135.9, 133.4, 128.5, 125.0, 119.7, 114.1, 47.1, 40.8, 36.1, 26.1. HRMS (ES+)
154 calcd for $C_{14}H_{13}N_4O_2S$ $(M + H)^+$, 329.0821, found, 329.0823.

155 *N-(3-(3-(7-Chloro-4-oxobenzo[d][1,2,3]triazin-3(4H)-yl)propyl)-4-oxothiazolidin*
156 *-2-ylidene)cyanamide (**VI-2**)*: yield, 52%; mp 163.4–164.2 ºC; ^{1}H NMR (400 MHz,
157 DMSO-d_6) δ 8.35 (d, J = 2.0 Hz, 1H), 8.25 (dd, J_1 = 8.4 Hz, J_2 = 2.0 Hz, 1H), 7.97
158 (dd, J_1 = 8.4 Hz, J_2 = 2.0 Hz, 1H), 4.40 (t, J = 7.2 Hz, 2H), 4.29 (s, 2H), 3.74 (t, J =
159 7.2 Hz, 2H), 2.20–2.09 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 179.6, 172.9,
160 154.8, 144.9, 140.3, 133.6, 127.7, 127.3, 118.6, 114.0, 47.3, 40.8, 36.1, 26.0. HRMS
161 (ES+) calcd for $C_{14}H_{11}N_6O_2NaS^{35}Cl$ $(M + Na)^+$, 385.0250, found, 385.0258; calcd
162 for $C_{14}H_{11}N_6O_2NaS^{37}Cl$ $(M + Na)^+$, 387.0221, found, 387.0232.

N-(3-(3-(8-Bromo-4-oxobenzo[d][1,2,3]triazin-3(4H)-yl)propyl)-4-oxothiazolidin-2-ylidene)cyanamide (***VI-3***): yield, 35%; mp 159.7–160.6 ºC; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.38 (d, J = 7.6 Hz, 1H), 8.24 (d, J = 7.6 Hz, 1H), 7.82 (t, J = 7.6 Hz, 1H), 4.41 (t, J = 6.8 Hz, 2H), 4.30 (s, 2H), 3.75 (t, J = 6.8 Hz, 2H), 2.26–2.07 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 179.6, 173.0, 154.5, 141.4, 139.4, 134.2, 124.9, 122.9, 121.8, 114.1, 47.5, 40.8, 36.1, 26.0. HRMS (ES+) calcd for $C_{14}H_{12}N_6O_2S^{79}Br$ $(M + H)^+$, 406.9926, found, 406.9927; calcd for $C_{14}H_{12}N_6O_2S^{81}Br$ $(M + H)^+$, 408.9905, found, 408.9908.

N-(3-(3-(7-Fluoro-4-oxobenzo[d][1,2,3]triazin-3(4H)-yl)propyl)-4-oxothiazolidin-2-ylidene)cyanamide (***VI-4***): yield, 32%; mp 171.1–171.6 ºC; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.34 (dd, J_1 = 8.8 Hz, J_2 = 6.0 Hz, 1H), 8.09 (dd, J_1 = 9.2 Hz, J_2 = 2.4 Hz, 1H), 7.82 (td, J_1 = 8.8 Hz, J_2 = 2.4 Hz, 1H), 4.41 (t, J = 7.2 Hz, 2H), 4.29 (s, 2H), 3.74 (t, J = 7.2 Hz, 2H), 2.23–2.07 (m, 2H); ^{19}F NMR (376 MHz, DMSO-d_6) δ -101.8 (td, J_1 = 9.0 Hz, J_2 = 5.6 Hz). HRMS (ES–) calcd for $C_{14}H_{10}N_6O_2FS$ $(M - H)^-$, 345.0570, found, 345.0570.

N-(3-(3-(6-(Trifluoromethyl)-4-oxobenzo[d][1,2,3]triazin-3(4H)-yl)propyl)-4-oxothiazolidin-2-ylidene)cyanamide (***VI-5***): yield, 31%; mp 174.6–175.4 ºC; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.50 (s, 1H), 8.46–8.38 (m, 2H), 4.44 (t, J = 7.2 Hz, 2H), 4.30 (s, 2H), 3.75 (t, J = 7.2 Hz, 2H), 2.23–2.11 (m, 2H); ^{19}F NMR (376 MHz, DMSO-d_6) δ -61.5 (s). HRMS (ES+) calcd for $C_{15}H_{11}N_6O_2F_3NaS$ $(M + Na)^+$, 419.0514, found, 419.0519.

*N-(3-(3-(8-Methyl-4-oxobenzo[d][1,2,3]triazin-3(4H)-yl)propyl)-4-oxothiazolidin-2-ylidene)cyanamide (**VI-6**)*: yield, 34%; mp 166.5–167.3 ºC; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.07 (d, J = 7.6 Hz, 1H), 7.91 (d, J = 7.2 Hz, 1H), 7.80 (t, J = 7.6 Hz, 1H), 4.41 (t, J = 7.2 Hz, 2H), 4.29 (s, 2H), 3.73 (t, J = 7.2 Hz, 2H), 2.77 (s, 3H), 2.23–2.09 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 179.6, 172.9, 155.4, 142.4, 137.7, 136.5, 133.1, 122.6, 119.8, 114.0, 47.1, 40.9, 36.1, 26.1, 17.1. HRMS (ES+) calcd for $C_{15}H_{15}N_6O_2S$ $(M + H)^+$, 343.0977, found, 343.0979.

*N-(3-(3-(8-Methoxy-4-oxobenzo[d][1,2,3]triazin-3(4H)-yl)propyl)-4-oxothiazolidin-2-ylidene)cyanamide (**VI-7**)*: yield, 40%; mp 190.0–190.9 ºC; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.86 (t, J = 8.0 Hz, 1H), 7.75 (d, J = 7.6 Hz, 1H), 7.63 (d, J = 8.0 Hz, 1H), 4.39 (t, J = 7.2 Hz, 2H), 4.29 (s, 2H), 4.04 (s, 3H), 3.72 (t, J = 7.2 Hz, 2H), 2.22–2.04 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 179.6, 172.9, 155.9, 155.1, 134.5, 134.4, 120.8, 116.8, 115.5, 114.1, 57.0, 47.1, 40.8, 36.1, 26.1. HRMS (ES–) calcd for $C_{15}H_{13}N_6O_3S$ $(M - H)^-$, 357.0770, found, 357.0769.

*N-(3-(3-(5-Bromo-4-oxobenzo[d][1,2,3]triazin-3(4H)-yl)propyl)-4-oxothiazolidin-2-ylidene)cyanamide (**VI-8**)*: yield, 39%; mp 201.5–202.5 ºC; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.18 (d, J = 8.0 Hz, 1H), 8.14 (d, J = 7.2 Hz, 1H), 7.91 (t, J = 8.0 Hz, 1H), 4.35 (t, J = 7.2 Hz, 2H), 4.31 (s, 2H), 3.75 (t, J = 7.2 Hz, 2H), 2.22–2.07 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 179.6, 173.0, 153.5, 145.7, 138.7, 136.1, 128.7, 119.2, 118.1, 114.1, 47.6, 40.9, 36.1, 25.9. HRMS (ES–) calcd for $C_{14}H_{10}N_6O_2S^{79}Br$ $(M - H)^-$, 404.9769, found, 404.9773; calcd for $C_{14}H_{10}N_6O_2S^{81}Br$

205 $(M - H)^-$, 406.9749, found, 406.9753.

206 *N-(3-(3-(6-Nitro-4-oxobenzo[d][1,2,3]triazin-3(4H)-yl)propyl)-4-oxothiazolidin-2*

207 *-ylidene)cyanamide (**VI-9**)*: yield, 46%; mp 186.0–187.8 ºC; ^{1}H NMR (400 MHz,

208 DMSO-d_6) δ 8.86 (d, J = 2.4 Hz, 1H), 8.78 (dd, J_1 = 8.8 Hz, J_2 = 2.0 Hz, 1H), 8.47

209 (d, J = 9.2 Hz, 1H), 4.45 (t, J = 6.8 Hz, 2H), 4.30 (s, 2H), 3.76 (t, J = 6.8 Hz, 2H),

210 2.27–2.12 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 179.7, 173.0, 154.6, 149.2,

211 146.4, 130.8, 129.8, 121.0, 120.6, 114.1, 47.7, 40.8, 36.1, 26.0. HRMS (ES–) calcd

212 for $C_{14}H_{10}N_7O_4S$ $(M - H)^-$, 372.0515, found, 372.0519.

213 *N-(3-(3-(7-Bromo-4-oxobenzo[d][1,2,3]triazin-3(4H)-yl)propyl)-4-oxothiazolidin*

214 *-2-ylidene)cyanamide (**VI-10**)*: yield, 41%; mp 180.9–181.0 ºC; ^{1}H NMR (400 MHz,

215 DMSO-d_6) δ 8.49 (s, 1H), 8.17 (d, J = 8.4 Hz, 1H), 8.10 (dd, J_1 = 8.4 Hz, J_2 = 1.6

216 Hz, 1H), 4.40 (t, J = 7.2 Hz, 2H), 4.29 (s, 2H), 3.73 (t, J = 7.2 Hz, 2H), 2.20–2.08

217 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 179.6, 173.0, 154.9, 144.9, 136.3, 130.7,

218 129.2, 127.3, 118.9, 114.1, 47.3, 40.8, 36.1, 26.0. HRMS (ES–) calcd for

219 $C_{14}H_{10}N_6O_2S^{79}Br$ $(M - H)^-$, 404.9769, found, 404.9780; calcd for $C_{14}H_{10}N_6O_2S^{81}Br$

220 $(M - H)^-$, 406.9749, found, 406.9760.

221 **Assay of Nematicidal Activity.** The second-stage juveniles (J2) of *M. incognita*

222 used in all tests were cultured by Huzhou Modern Agricultural Biotechnology

223 Innovation Center, Chinese Academy of Sciences, China.

224 *In vivo.* All compounds (**VI-1**–**23**) were dissolved with DMF and diluted with

225 distilled water to obtain series concentrations of 40.0, 25.0, 10.0, 5.0, and 1.0 mg L^{-1}

for bioassays. The final concentration of DMF in each treatment never exceeded 1% v/v. The one-week age cucumber seedlings were replanted in sterilized sand in test tubes (one seedling per test tube, tube size: 20 × 250 mm), and the roots of each seedling were treated with 3 mL of test solution. Then approximately 2000 living J2 nematodes were inoculated into the rhizosphere sand of each host plant. Fenamiphos and avermectin (B1) at concentrations of 5.0 and 1.0 mg L^{-1} served as positive control, and the negative control group was prepared in the same way but lacked the tested compound. Distilled water without nematodes served as blank control. Each treatment was replicated four times and the experiment was repeated three times. All the above test tubes were incubated at 20–25 ºC for 20 days, with 10 h in the daylight and 14 h in the dark per day. The number of root knots in each test tube was counted and recorded a score. The inhibition rate on J2 of *M. incognita* was calculated by comparison with the negative control group:

Inhibition rate (%) = [(score of negative control – score of treatment) / (score of negative control)] × 100.

Scoring criteria:

0: 0–5 knots; 5: 6–10 knots; 10: 11–20 knots; 20: more than 20 knots.

In vitro. Pure compounds (**VI-1**–**23**) were dissolved in DMF and diluted with distilled water to obtain stock solutions of double the treatment concentration. Then 2 mL of J2 aqueous suspension containing approximately 200 living nematodes was added onto a 6 cm diameter Petri dish, and treated with 2 mL of the above solution,

247 providing series concentrations of 50.0, 25.0, 10.0, 5.0 and 1.0 mg L^{-1} in the
248 meanwhile. The final concentration of DMF in each treatment never exceeded 1%
249 v/v. Avermectin (B1) at the above same treatment concentrations served as positive
250 control, and the negative control group was prepared in the same way but lacked the
251 tested compound. Distilled water served as blank control. All the above test dishes
252 were covered with the laboratory parafilm to avoid the possible evaporation or
253 pollution. Each treatment was incubated at 25 ºC for 24 hours and had three
254 repetitions. Nematodes in each test dish were collected after washing in sterile water
255 through a 500-mesh sieve, and finally the activities of tested compounds were
256 monitored under a microscope by recording the death rates of tested nematodes.
257 Nematodes that did not move when prodded with a needle were considered to be
258 dead. The LC_{50} values of tested compounds were calculated using the probit method.

259 **RESULTS AND DISCUSSION**

260 **Synthesis.** Starting from anthranilic acids (**I-1**–**13**), the desired isatoic anhydrides
261 (**II-1**–**13**) were readily prepared via the annulation reaction with BTC.[32] As shown in
262 Figure 4, various substituents at nearly every position of the benzene ring were well
263 tolerated except 5-NO_2 and 8-NO_2. 5-NO_2 or 8-NO_2 isatoic anhydride could not be
264 obtained from the corresponding anthranilic acid due to the possible formation of
265 intramolecular hydrogen bond between NO_2 and adjacent carboxyl or amino.
266 Subsequently, **II-1**–**13** reacted with ammonium to afford anthranilamides[33] (**III-1**–
267 **13**). Notably, ammonium carbonate was utilized here as ammonium source, which

made synthetic manipulation relatively easier. 1,2,3-Benzotriazin-4-ones (**IV-1**–**13**) were synthesized from **III-1**–**13** through the combination of diazotization, nucleophilic addition and cyclization in one pot.[34]

The synthesis of 3-bromoalkyl-1,2,3-benzotriazin-4-ones (**V-1**–**13**) was carried out through *N*-alkylation of **IV-1**–**13** at 3-position, using dibromoalkane as alkylation agent[36] (Figure 6). To minimize the 'bis-' product, 5 equiv. of dibromoalkane was necessary, owing to the existing two reaction sites of dibromoalkane. Potassium salt of 2-cyanoimino-4-oxothiazolidine was prepared following the procedure reported in the literature[35] (Figure 5). The potassium salt of 2-cyanoimino-4-oxothiazolidine has higher nucleophilic activity than 2-cyanoimino-4-oxothiazolidine, which promoted the nucleophilic substitution. Then the title compounds (**VI-1**–**23**) were synthesized by the reaction of 3-bromoalkyl-1,2,3-benzotriazin-4-ones with potassium salt of 2-cyanoimino-4-oxothiazolidine in the presence of potassium iodide (Figure 6). The structures of the title compounds were well characterized by ^{1}H NMR, ^{13}C NMR and HR-MS (ESI).

Nematicidal Activity. As shown in Table 1, the *in vivo* nematicidal activities of title compounds (**VI-1**–**23**) against *M. incognita* were initially evaluated at the concentration of 40.0 mg L^{-1}. Some compounds such as **VI-1**, **VI-3**, **VI-6** and **VI-7** showed high nematicidal activities with inhibition rates of 100%, whereas others were severely toxic to plants with a symptom of root rot. To minimize the

289 phytotoxicity of test compounds, a lower treatment concentration was tried. When
290 treated at the concentration of 25.0 mg L^{-1}, the phytotoxicity was significantly
291 improved and quite a few compounds exhibited moderate to high nematicidal
292 activities. Among them, compounds **VI-2**, **VI-5**, **VI-8**, **VI-14**, **VI-20**, and **VI-23**
293 were 100% inhibitory against *M. incognita*. When the treatment concentration was
294 reduced further to 10.0 mg L^{-1}, all compounds were nematicidal without
295 phytotoxicity, and compounds **VI-2**, **VI-9**, **VI-14**, **VI-20**, and **VI-23** still keep 100%
296 inhibitory activities.

297 On the basis of the *in vivo* data at 10.0 mg L^{-1} in Table 1, it was found that
298 compounds **VI-2** (7-Cl, n=1), **VI-9** (6-NO_2, n=1), **VI-14** (7-Cl, n=0), **VI-20** (7-NO_2,
299 n=4), and **VI-23** (7-OCH_3, n=4) still exhibited 100% inhibitory rates. But we did not
300 observe the obvious trend with electron withdrawing or donating substituents. When
301 Cl, NO_2, OCH_3 were located at 7-position (**VI-2**, **VI-14**, **VI-19**, **VI-20**, and **VI-23**)
302 and CF_3, NO_2 at 6-position (**VI-5**, **VI-9**), or Br at 5-position (**VI-8**) on the
303 1,2,3-benzotriazin-4-one ring, compounds with proper linkers displayed >90%
304 inhibitory activities. It indicated that high inhibitory activity seemed not to depend
305 upon the type of substituents on the 1,2,3-benzotriazin-4-one ring. When the linker
306 was fixed as a three-carbon chain, the activity of compound **VI-2** (7-Cl) was the
307 highest among the compounds (**VI-1**–**13**) considering that compound **VI-9** (6-NO_2)
308 had the phytotoxicity at 25.0 mg L^{-1}. Moreover, for other compounds with a
309 three-carbon chain, it showed that compound **VI-8** (5-Br) had higher activity than

compounds **VI-3** (8-Br) and **VI-10** (7-Br), and compounds **VI-6** (8-CH_3), **VI-7** (8-OCH_3) and **VI-9** (6-NO_2) were more active than compounds **VI-11** (6-CH_3), **VI-13** (7-OCH_3) and **VI-12** (7-NO_2), respectively. Thus it seemed that the inhibitory activity was affected by the co-effect of substituent type and substituted position on the premise that the length of flexible linker was fixed.

Furthermore, when the NO_2 or OCH_3 group was fixed at 7-position, the effect of linker length on inhibitory activity was investigated. It was observed that the activities of compounds did not present a certain regularity with the increasing of linker length, except compound **VI-13**. But for Cl at the same substituted position, the activities did not present a certain regularity with the increasing of linker length. Thus, it also suggested that inhibitory activities indeed had no positive correlation with linker length. From the results above, it can be concluded preliminarily that the inhibitory activity of our synthesized compounds was influenced by the combination of substituent type, substituted position and linker length, not relying on only one factor. It is different from the results reported by Che *et al* and Li *et al*.[37,38] Che *et al* reported that a shorter chain length of R^3 and electron deficiency of the indolyl ring were favorable to nematicidal activity,[37] while Li *et al* reported that electron richness of the benzyl ring was favorable to nematicidal activity.[38]

To determine the strength of activity of title compounds, the nematicidal evaluation of the compounds with >90% inhibitory activities at 10.0 mg L^{-1} such as **VI-2**, **VI-5**, **VI-8**, **VI-9**, **VI-14**, **VI-19**, **VI-20** and **VI-23** was continued at lower

concentrations of 5.0 and 1.0 mg L^{-1}, but it was found that the inhibitory activities decreased very quickly with the decreasing of the treatment concentration. All of the inhibitory rates were less than 41% at these two concentrations. The reason why the inhibitory rates decreased so quickly still needs to be investigated further.

In addition, the *in vitro* nematicidal evaluation of compounds **VI-1**–**23** against *M. incognita* was conducted. As positive control, avermectin had the LC_{50} of 1.0 ± 0.1 mg L^{-1}. Unexpectedly, all synthesized compounds showed only <5% corrected mortalities at 50.0 mg L^{-1} (not listed here), which indicated that these compounds had no direct inhibition against *M. incognita*. The significant difference between *in vivo* and *in vitro* data attracted our attention. One possible reason is that these compounds could not kill the nematodes directly like some nervous toxicants such as avermectin but affect the movement and host finding of nematodes,[39,40] or they could change the plant's rhizosphere microbiology strengthening antagonism against nematodes,[41] somewhat like the DiTera nematicide. Further research is still needed to verify the possible mechanism.

In conclusion, a series of novel 1,2,3-benzotriazin-4-one derivatives bearing 2-cyanothiazolidin-4-one were synthesized, and both *in vivo* and *in vitro* nematicidal activities against *M. incognita* were evaluated. Despite no direct inhibition *in vitro*, some of them exhibited good *in vivo* inhibitory activities at 10.0 mg L^{-1}, which implied that 1,2,3-benzotriazin-4-one is a potential active structure worth to study further.

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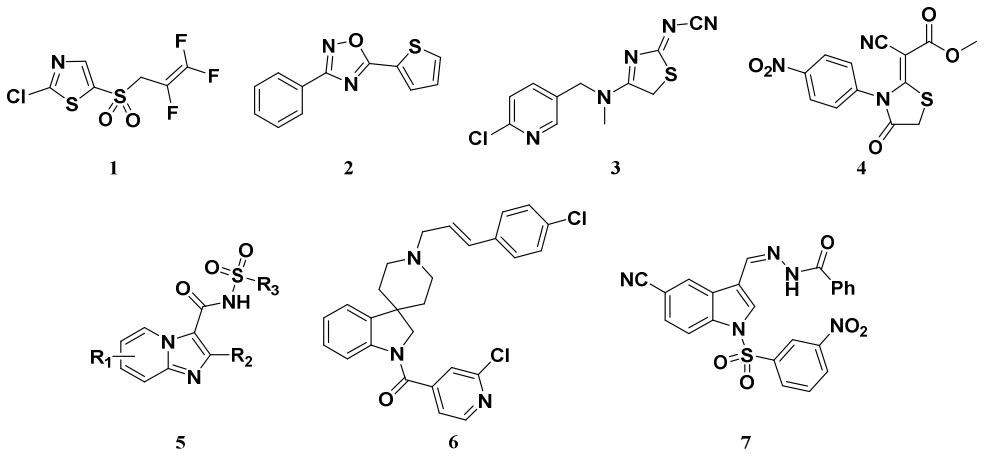
Figure 6. Synthetic route of title compounds (VI-1–23).



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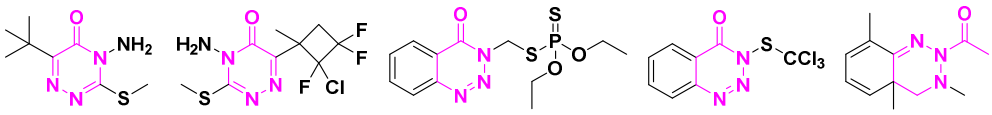
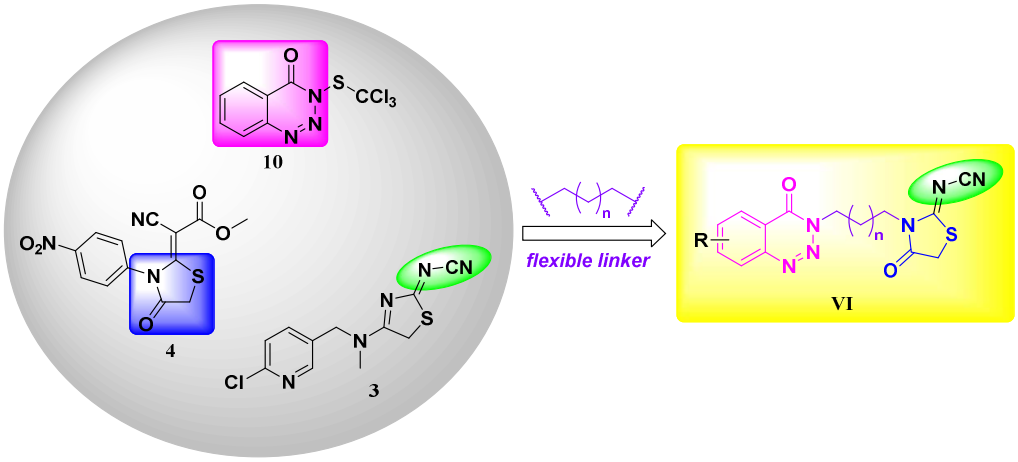


Figure 2. Bioactive agrochemicals containing triazinone skeletons.



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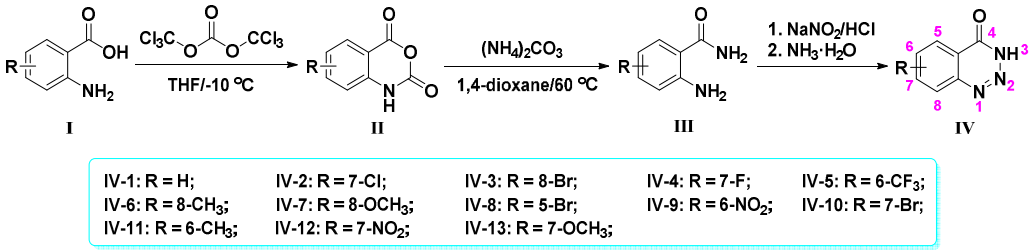

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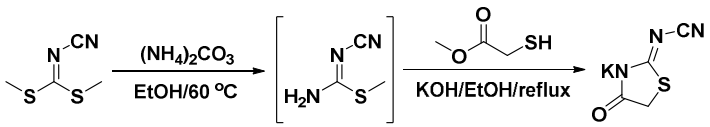
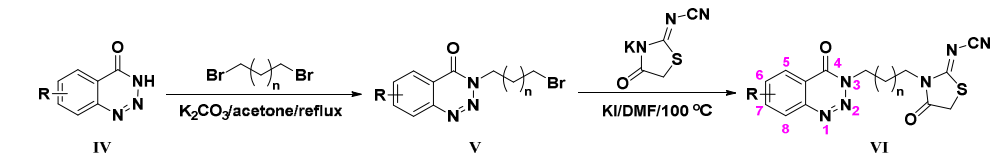


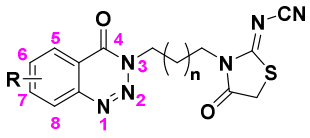
Figure 5. Synthetic route of potassium salt of 2-cyanoimino-4-oxothiazolidine.



VI-1: R = H, n = 1; VI-2: R = 7-Cl, n= 1; VI-3: R = 8-Br, n = 1; VI-4: R = 7-F, n = 1; VI-5: R = 6-CF_3, n = 1;
VI-6: R = 8-CH_3, n = 1; VI-7: R = 8-OCH_3, n = 1; VI-8: R = 5-Br, n = 1; VI-9: R = 6-NO_2, n = 1; VI-10: R = 7-Br, n = 1;
VI-11: R = 6-CH_3, n = 1; VI-12: R = 7-NO_2, n = 1; VI-13: R = 7-OCH_3, n = 1; VI-14: R = 7-Cl, n= 0; VI-15: R = 7-Cl, n= 2;
VI-16: R = 7-Cl, n= 3; VI-17: R = 7-Cl, n= 4; VI-18: R = 7-NO_2, n = 2; VI-19: R = 7-NO_2, n = 3; VI-20: R = 7-NO_2, n = 4;
VI-21: R = 7-OCH_3, n = 2; VI-22: R = 7-OCH_3, n = 3; VI-23: R = 7-OCH_3, n = 4

Figure 6. Synthetic route of title compounds (**VI-1**–**23**).

Table 1. Control Efficacy of Compounds VI-1–23 against the Cucumber Root-knot Nematode Disease Caused by *M. incognita* in Test Tubes



VI

Compd.	R	n	J2 of *M. incognita* Inhibition rate (%)				
			40.0 mg L^{-1}	25.0 mg L^{-1}	10.0 mg L^{-1}	5.0 mg L^{-1}	1.0 mg L^{-1}
VI-1	H	1	100	76.5	58.3		
VI-2	7-Cl	1	pt[c]	100	100	38.7	27.2
VI-3	8-Br	1	100	78.0	63.5		
VI-4	7-F	1	pt	pt	74.5		
VI-5	6-CF_3	1	pt	100	90.8	31.5	19.4
VI-6	8-CH_3	1	100	88.7	67.3		
VI-7	8-OCH_3	1	100	88.1	66.7		
VI-8	5-Br	1	pt	100	91.8	34.0	20.3
VI-9	6-NO_2	1	pt	pt	100	37.2	23.9
VI-10	7-Br	1	pt	91.5	70.6		
VI-11	6-CH_3	1	pt	62.7	41.1		
VI-12	7-NO_2	1	pt	pt	64.3		
VI-13	7-OCH_3	1	pt	pt	54.0		
VI-14	7-Cl	0	pt	100	100	36.4	20.0
VI-15	7-Cl	2	pt	pt	53.6		
VI-16	7-Cl	3	pt	pt	26.1		
VI-17	7-Cl	4	pt	pt	63.4		
VI-18	7-NO_2	2	pt	pt	71.4		
VI-19	7-NO_2	3	pt	pt	90.1	30.1	17.6
VI-20	7-NO_2	4	pt	100	100	37.2	22.2
VI-21	7-OCH_3	2	pt	pt	38.9		
VI-22	7-OCH_3	3	pt	pt	83.3		
VI-23	7-OCH_3	4	pt	100	100	40.5	30.6
FM[a]						100	100
AVM[b]						100	100

[a] **FM**: fenamiphos; [b] **AVM**: avermectin; [c] pt: phytotoxic.

TOC graphic