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PII: S0960-894X(16)31345-2
DOI: http://dx.doi.org/10.1016/j.bmcl.2016.12.065
Reference: BMCL 24554

To appear in: *Bioorganic & Medicinal Chemistry Letters*

Received Date: 29 October 2016
Revised Date: 9 December 2016
Accepted Date: 27 December 2016

Please cite this article as: Chang, Y., Zhang, J., Chen, X., Li, Z., Xu, X., Synthesis and Nematicidal Activities of 1,2,3-Benzotriazin-4-one Derivatives Containing Thiourea and Acylthiourea against *Meloidogyne incognita*, *Bioorganic & Medicinal Chemistry Letters* (2016), doi: http://dx.doi.org/10.1016/j.bmcl.2016.12.065

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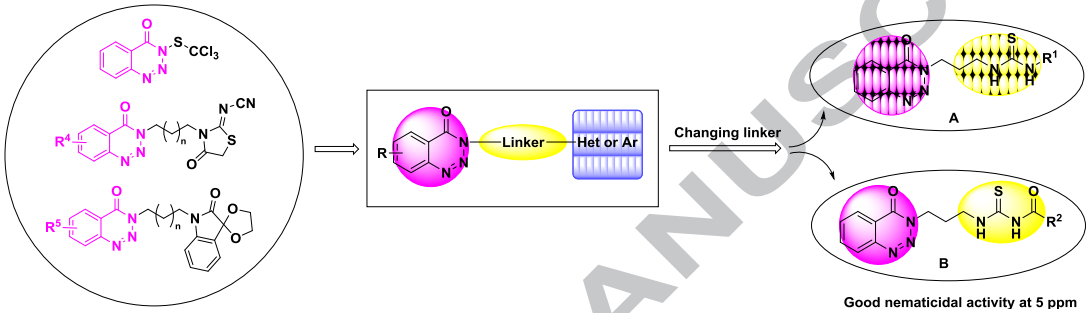

Bioorganic & Medicinal Chemistry Letters
journal homepage: www.elsevier.com

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

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ARTICLE INFO

Article history:
Received
Revised
Accepted
Available online

Keywords:
1,2,3-Benzotriazin-4-one
Thiourea
Acylthiourea
M. incognita
Nematicidal activity

ABSTRACT

Two series of novel 1,2,3-benzotriazin-4-one derivatives containing thiourea and acylthiourea were designed and synthesized. The bioassay results showed that most of the test compounds showed good nematicidal activity against *M. incognita* at the concentration of 10.0 mg L^{-1} *in vivo*. The compounds **A13**, **A17** and **B3** showed excellent nematicidal activity on the second stage juveniles of the root-knot nematode with the inhibition rate of 51.3%, 58.3% and 51.3% at the concentration of 1.0 mg L^{-1} respectively. It suggested that the structure of 1,2,3-benzotriazin-4-one derivatives containing thiourea and acylthiourea could be optimized further.

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Plant-parasitic nematodes (PPNs) cause approximately $157 billion of annual crop losses globally.[1] Among them, root-knot nematodes (RKNs), *Meloidogyne* spp., are considered as the most damaging nematode group in the world as they result in approximately 5% of global crop loss to most cultivated plant species such as tomato, pepper, watermelons and onions.[2-4] Traditional nematocides such as fosthiazate, fenamiphos, oxamyl, dazomet, 1,3-dichloropropene and metham sodium have been applied for many years. However, some of these chemicals are gradually being phased out because of their bad environmental impact. At present, fosthiazate and abamectin are the most commonly used nematocides in the market. Although recently some stars of nematocide industry have entered the pesticide market like fluensulfone[5-7] and tioxazafen[8] (Figure 1), it is not enough for the increasingly stringent regulatory requirements for protecting the environment and ensuring food safety. Therefore, it is urgent to develop environment-friendly alternatives for PPNs control.

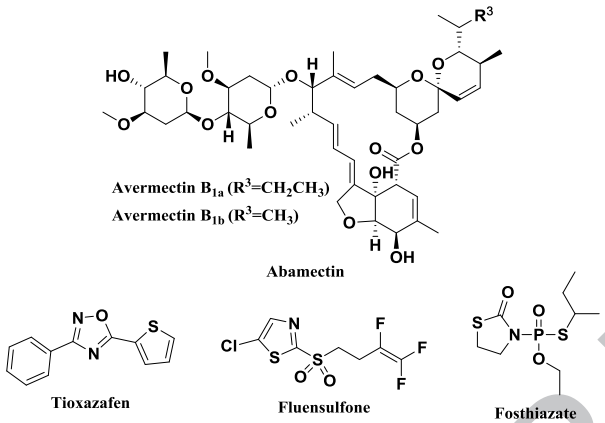


Figure 1. Some representative nematicides.

1,2,3-Benzotriazine-4-one is an important class of nitrogen-containing heterocycle and has attracted much attention in both the medicinal and agrochemical fields.[9-11] For example, many pharmacological properties for this class of compounds have been reported, including drugs with sedative, anesthetic, antitumor, antiarthritic, diuretic and antitubercular activities.[12-17] 1,2,3-Benzotriazine-4-one structure has also been applied on organophosphorus insecticide such as azinphos-ethyl and azinphos-methyl, which can be used for crops pests prevention and control. As shown in Figure 2, the 1,2,3-benzotriazin-4-one derivative also exhibited nematicidal activity. Compound **V1** could inhibit *Anguillula* nematodes.[18] In addition, our research group have reported that 1,2,3-benzotriazin-4-one derivatives **V2** and **V3** exhibited good control efficacy against the cucumber root-knot nematode disease caused by *Meloidogyne incognita* at the concentration of 10.0 mg L^{-1}, which implied that 1,2,3-benzotriazin-4-one derivatives might be potential for novel promising nematicides.[19,20] Analyzing the structure characteristic of these lead compounds, we found that the structure includes three parts: 1,2,3-benzotriazin-4-one, linker, heterocycle or aromatic ring. The trial of changing heterocycle and the effect of substituents on 1,2,3-benzotriazin-4-one ring have been investigated before[19,20], so we focus our attention on the change of linker now. In crop protection and bioactive chemicals, thiourea and acylthiourea have been reported to display a variety of biological activities, such as insecticidal, fungicidal, antimicrobial, antitumor, etc.[21-26] With all this in mind, we introduced thiourea and acylthiourea into 1,2,3-benzotriazin-4-one structure as linker to investigate the effect of the linker type on the nematicidal bioactivity, and designed two series of novel 1,2,3-benzotriazin-4-one derivatives (Fig.2 **A** and **B**). Herein, we described the molecular design, synthesis and preliminarily discussion about the relationship between structure and nematicidal activities against *Meloidogyne incognita in vivo*.

1,2,3-Benzotriazin-4-one (**2**) was prepared according to the method in reported literature.[27] As depicted in Scheme 1, the compound **3** was readily prepared via N-alkylation of 1,2,3-Benzotriazin-4-one at the 3 position, with 2-(3-bromopropyl)isoindoline-1,3-dione as an alkylation agent. Subsequently, the compound **3** was hydrolysis to 3-(3-aminopropyl)benzo[d][1,2,3]triazin-4(3H)-one (**4**) in the presence of hydrazine through Gabriel's primary amine synthesis.

As shown in Scheme 2, arylamines **5** reacted with CS_2 under the organic base (triethylene diamine) condition to afford intermediates **6**. Then intermediates **6** reacted with triphosgene in $CHCl_3$ to produce aryl isothiocyanates (**7**). Benzoyl chlorides (**9**) were readily prepared via chloroformylation reaction. Then, aroyl isothiocyanates (**10**) were synthesized by benzoyl chlorides reacted with ammonium thiocyanate under the condition of PEG-400 as phase transfer catalyst. Finally, the title compounds (**A1**−**A17**) were synthesized by the reaction of aryl isothiocyanates (**7**) with intermediate **4** in acetonitrile (Scheme 1). And,aroyl isothiocyanates (**10**) reacted with amine (**4**) to produce the title compounds **B1**−**B11** (Scheme 1). The structures of the title compounds were well-characterized by ^{1}H NMR, ^{13}C NMR, IR, and HRMS (ESI).

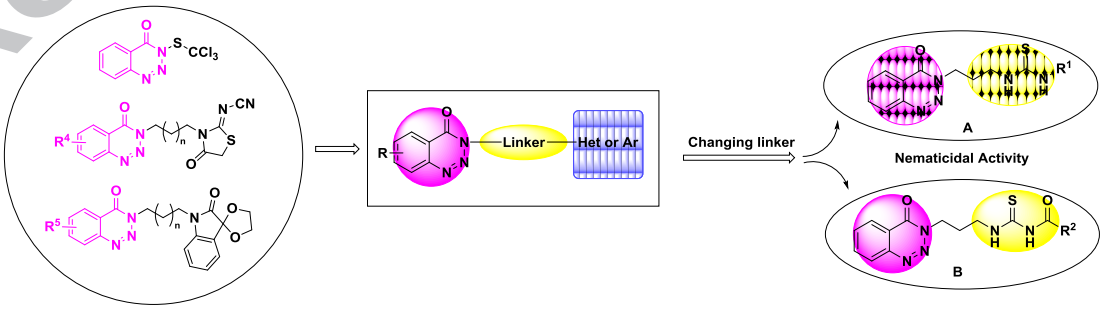
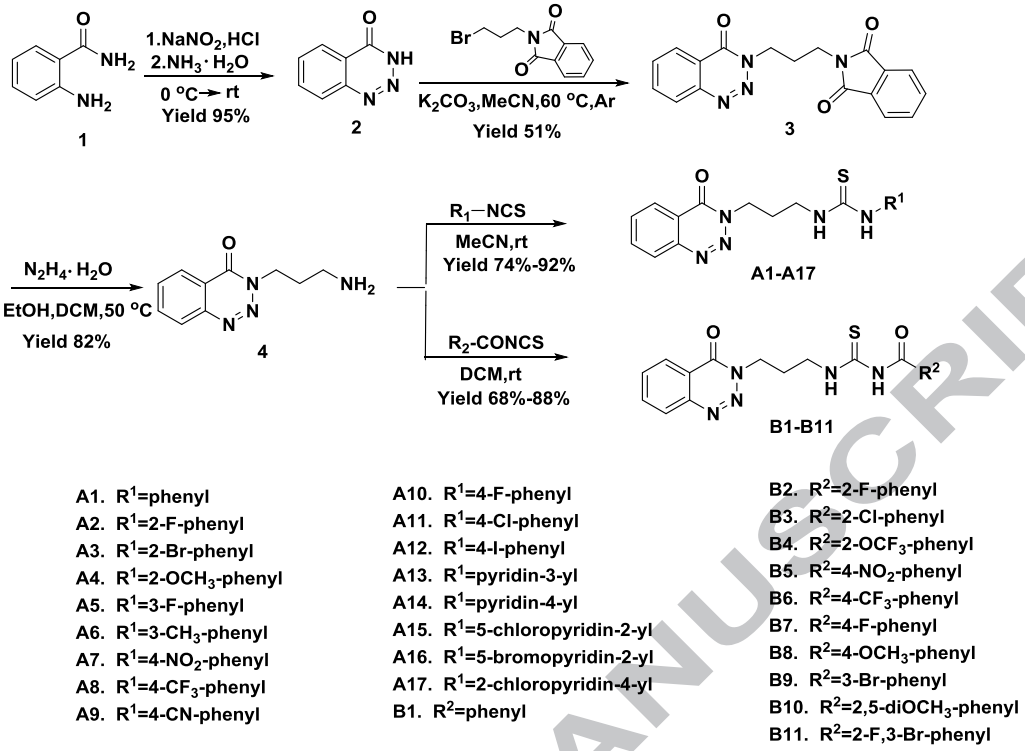
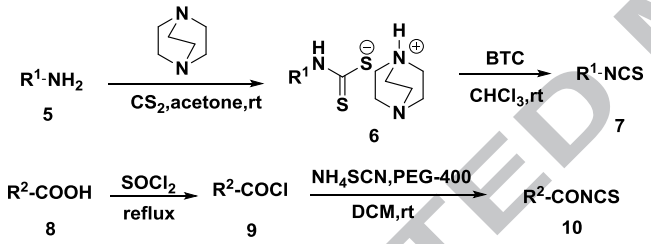


Figure 2. Design strategy for the synthesis of 1,2,3-benzotriazin-4-one derivatives containing thiourea and acylthiourea.



A1. R^1=phenyl
A2. R^1=2-F-phenyl
A3. R^1=2-Br-phenyl
A4. R^1=2-OCH_3-phenyl
A5. R^1=3-F-phenyl
A6. R^1=3-CH_3-phenyl
A7. R^1=4-NO_2-phenyl
A8. R^1=4-CF_3-phenyl
A9. R^1=4-CN-phenyl
A10. R^1=4-F-phenyl
A11. R^1=4-Cl-phenyl
A12. R^1=4-I-phenyl
A13. R^1=pyridin-3-yl
A14. R^1=pyridin-4-yl
A15. R^1=5-chloropyridin-2-yl
A16. R^1=5-bromopyridin-2-yl
A17. R^1=2-chloropyridin-4-yl
B1. R^2=phenyl
B2. R^2=2-F-phenyl
B3. R^2=2-Cl-phenyl
B4. R^2=2-OCF_3-phenyl
B5. R^2=4-NO_2-phenyl
B6. R^2=4-CF_3-phenyl
B7. R^2=4-F-phenyl
B8. R^2=4-OCH_3-phenyl
B9. R^2=3-Br-phenyl
B10. R^2=2,5-diOCH_3-phenyl
B11. R^2=2-F,3-Br-phenyl

Scheme 1. Synthetic route of title compounds (**A1**–**A17**, **B1**–**B11**).



Scheme 2. Synthetic route of aryl isothiocyanates and aroyl isothiocyanates.

As shown in Table 1, the *in vivo* nematicidal activities of aryl thiourea compounds (**A1**−**A17**) against *M. incognita* were initially evaluated at the concentration of 40.0 mg L^{-1}. Compound **A7** containing nitro group had high nematicidal activity with inhibition rate of 95.7%. Besides, most of the phenylthiourea compounds (**A1**−**A12**) showed <50% inhibitory activities against *M. incognita* and they exhibited phytotoxic to plants with a symptom of root rot to some extent. So the above compounds were not screening further. However, it was inspiring to found all the pyridylthiourea compounds (**A13**−**A17**) resulted in 100% inhibition activity when the benzene ring was replaced by pyridine ring. To minimize the phytotoxicity of test compounds, a lower treatment concentration was investigated. When the treatment concentration was reduced further to 20.0 mg L^{-1}, pyridylthiourea compounds (**A13**−**A17**) still exhibited good inhibitory effect on *M. incognita* among thiourea compounds. Especially, the inhibition rates of **A14** and **A15** reached 96.8% and 96.0%, respectively. Then, the nematicidal activities under 10.0, 5.0 and 1.0 mg L^{-1} treatment concentrations were investigated continually based on these results. It was found that even when the treatment concentration decreased to 1.0 mg L^{-1}, **A13** and **A17** kept better nematicidal activity with the inhibition rates of 51.3% and 58.3% respectively, which indicated the introduction of pyridine group is benefit for increasing bioactivity in this series.

For acylthiourea derivatives series, at the concentration of 40.0 mg L^{-1}, all compounds exhibited severely phytotoxic to plants. To minimize the phytotoxicity of test compounds, a lower treatment concentration was tried. When treated at the concentration of 20.0 mg L^{-1}, it is encouraging that acylthiourea derivatives **B** were >50% inhibitory activities against *M. incognita* except **B1**, and their range of inhibitory rates were between 79.7% and 97.8%. On the basis of the *in vivo* nematicidal activities data at 10.0 mg L^{-1} in Table 1, it was found that substitution on the benzene ring bearing either electron donating or withdrawing group irrespective of position enhanced the nematicidal activity for acylthiourea compounds. Compounds **B4** and **B8** were most active among all the test compounds with inhibition rates 89.9%, 83.8%, respectively. Compounds **B9** (81.7%), **B11** (80.5%) and **B2** (79.7%) were next in performance. However, we did not observe the obvious trend about the effect of electron-withdrawing or electron-donating substituents. When the treatment concentration continued to be reduced to 5.0 mg L^{-1}, the inhibition rate of compounds **B2**, **B3**, **B4**, **B7**, **B8**, **B10**, **B11** were still higher than 50%. Moreover, the inhibitory rate of **B3** reached 51.3% at 1.0 mg L^{-1}, which was valuable to investigate further for its greenhouse bioactivity.

Compared with the 1,2,3-benzotriazin-4-one derivatives containing thiourea, acylthiourea derivatives B exhibited better performance at nematicidal activity as a whole. For example, the inhibitory rates of compound **B2** and **B6** at 40.0 mg L^{-1} were 87.5% and 97.7% respectively, and they still kept a certain bioactivity at 1.0 mg L^{-1}; while compound **A2** and **A8** only had 38.7% and 79.6% inhibitory rates at 40.0 mg L^{-1}, respectively. The synthesis of acylthiourea derivatives with pyridine group is in progress.

In conclusion, two series of novel 1,2,3-benzotriazin-4-one derivatives containing thiourea and arylthiourea were synthesized, and *in vivo* nematicidal activities against *M. incognita* were evaluated at different treatments concentration.

Table 1. Nematicidal activities of Compounds **A1**–**17**, **B1**–**B11** against *M. incognita* in Test Tubes.

		J2 of *M. Incognita* inhibition rate (%)[c]				
Compd	**R^1/R^2**	**40.0 mg L^{-1}**	**20.0 mg L^{-1}**	**10.0 mg L^{-1}**	**5.0 mg L^{-1}**	**1.0mg L^{-1}**
A1	phenyl	38.7				
A2	2-F-phenyl	38.7				
A3	2-Br-phenyl	46.9				
A4	2-OCH_3-phenyl	56.5				
A5	3-F-phenyl	46.9				
A6	3-CH_3-phenyl	50.9				
A7	4-NO_2-phenyl	95.7	100	72.5	26.8	18.9
A8	4-CF_3-phenyl	79.6				
A9	4-CN-phenyl	45.6				
A10	4-F-phenyl	15.3				
A11	4-Cl-phenyl	10.1				
A12	4-I-phenyl	50.9				
A13	pyridin-3-yl	100	82.1	61.3	52.4	51.3
A14	pyridin-4-yl	100	96.8	79.2	35.1	0
A15	5-chloropyridin-2-yl	100	96.0	88.1	40.5	43.5
A16	5-bromopyridin-2-yl	100	58.3	51.3	46.4	40.5
A17	2-chloropyridin-4-yl	100	85.1	67.3	64.3	58.3
B1	phenyl	pt[d]	48.5	37.2	14.0	7.4
B2	2-F-phenyl	pt	87.5	79.7	59.4	26.9
B3	2-Cl-phenyl	pt	91.9	77.7	51.3	51.3
B4	2-OCF_3-phenyl	pt	97.8	89.9	71.6	39.1
B5	4-NO_2-phenyl	pt	83.8	65.4	42.6	45.1
B6	4-CF_3-phenyl	pt	97.7	61.0	47.2	33.4
B7	4-F-phenyl	pt	83.8	69.7	50.2	41.4
B8	4-OCH_3-phenyl	pt	91.9	83.8	73.6	24.9
B9	3-Br-phenyl	pt	84.8	81.7	41.2	26.9
B10	2,5-diOCH_3-phenyl	pt	79.7	71.6	63.2	20.9
B11	2-F,3-Br-phenyl	pt	93.5	80.5	51.3	2.6
[a]**AVM**					100	100
[b]**FM**					100	100

[a] AVM: avermectin. [b] FM: fenamiphos. [c] Average of three experiments. [d] phytotoxic

The inhibition rates of compounds **A13**, **A17**, **B3** were 51.3%, 58.3%, 51.3% at 1 mg L^{-1}, respectively. It suggested that these two structures were valuable to optimize further to find higher nematicidal activity leading compounds.

Acknowledgments

This work was financial supported by the National Natural Science Foundation of China (21672061).

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