Bioorganic & Medicinal Chemistry Letters 24 (2014) 2621–2624

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis and insecticidal activity of novel hydrazone compounds derived from a naturally occurring lignan podophyllotoxin against *Mythimna separata* (Walker)

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ARTICLE INFO

Article history:
Received 20 March 2014
Revised 18 April 2014
Accepted 19 April 2014
Available online 29 April 2014

Keywords:
Podophyllotoxin
Insecticidal activity
Botanical insecticide
Structural modification
Mythimna separata Walker

ABSTRACT

In continuation of our program aimed at the discovery and development of natural-product-based insecticidal agents, a series of novel hydrazone derivatives of podophyllotoxin, which is a naturally occurring aryltetralin lignan and isolated as the main secondary metabolite from the roots and rhizomes of *Podophyllum* species, were synthesized and evaluated as insecticidal agents against the pre-third-instar larvae of oriental armyworm, *Mythimna separata* (Walker) in vivo at 1 mg/mL. Especially compounds **8i**, **8j**, **8t**, and **8u** showed the more potent insecticidal activity with the final mortality rates greater than 60%.

Nowadays, synthetic chemical insecticides have played an important role in modern agricultural pest management, however, repeat application of those agrochemicals over the years has led to the development of resistance in insect pest populations and environmental problems.[1–3] On the other hand, as plant secondary metabolites result from the interaction between plants and environment (life and non-life) during the long period of evolution, pesticides produced from plant secondary metabolites may result in less or slower resistance development and lower pollution.[4] Recently, the discovery of new pesticidal agents from plant secondary metabolites, or by using them as the lead compounds for further structural modification, have been one of the important procedures for research and development of new insecticides.[5–10] Some pesticides from natural products such as nicotine, avermectins, azadirachtin, pyrethrum and neem extracts are characteristic examples as defenses against pests.[11–13]

Podophyllotoxin (**1**, Fig. 1), a naturally occurring aryltetralin lignan, is isolated from the roots and rhizomes of *Podophyllum hexandrum* such as *P. hexandrum* and *Podophyllum peltatum*. Besides its use as the lead compound for the preparation of potent anticancer drugs such as etoposide, teniposide and etopophos,[14–17] compound **1** has also received much research attention for its interesting insecticidal and antifungal activities.[18–23] More recently, we found

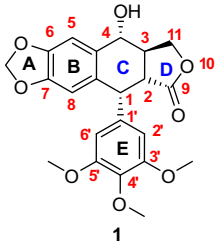

Figure 1. The chemical structure of podophyllotoxin (**1**).

that introduction of hydrazone fragments into fraxinellone or piperine could lead to more pronounced derivatives as compared with toosendanin, a commercial botanical insecticide isolated from *Melia azedarach*.[24,25] Encouraged by the above-mentioned interesting results, and in continuation of our program aimed at the discovery and development of novel natural-product-based pesticidal agents,[26–29] in this Letter we synthesized a series of novel podophyllotoxin-based hydrazone derivatives by introduction of hydrazone fragments at the C-9 position on the podophyllotoxin skeleton. Their insecticidal activity was tested against the pre-third-instar larvae of oriental armyworm, *Mythimna separata* (Walker) in vivo. In addition, their structure–activity relationships (SARs) were also described.

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http://dx.doi.org/10.1016/j.bmcl.2014.04.074
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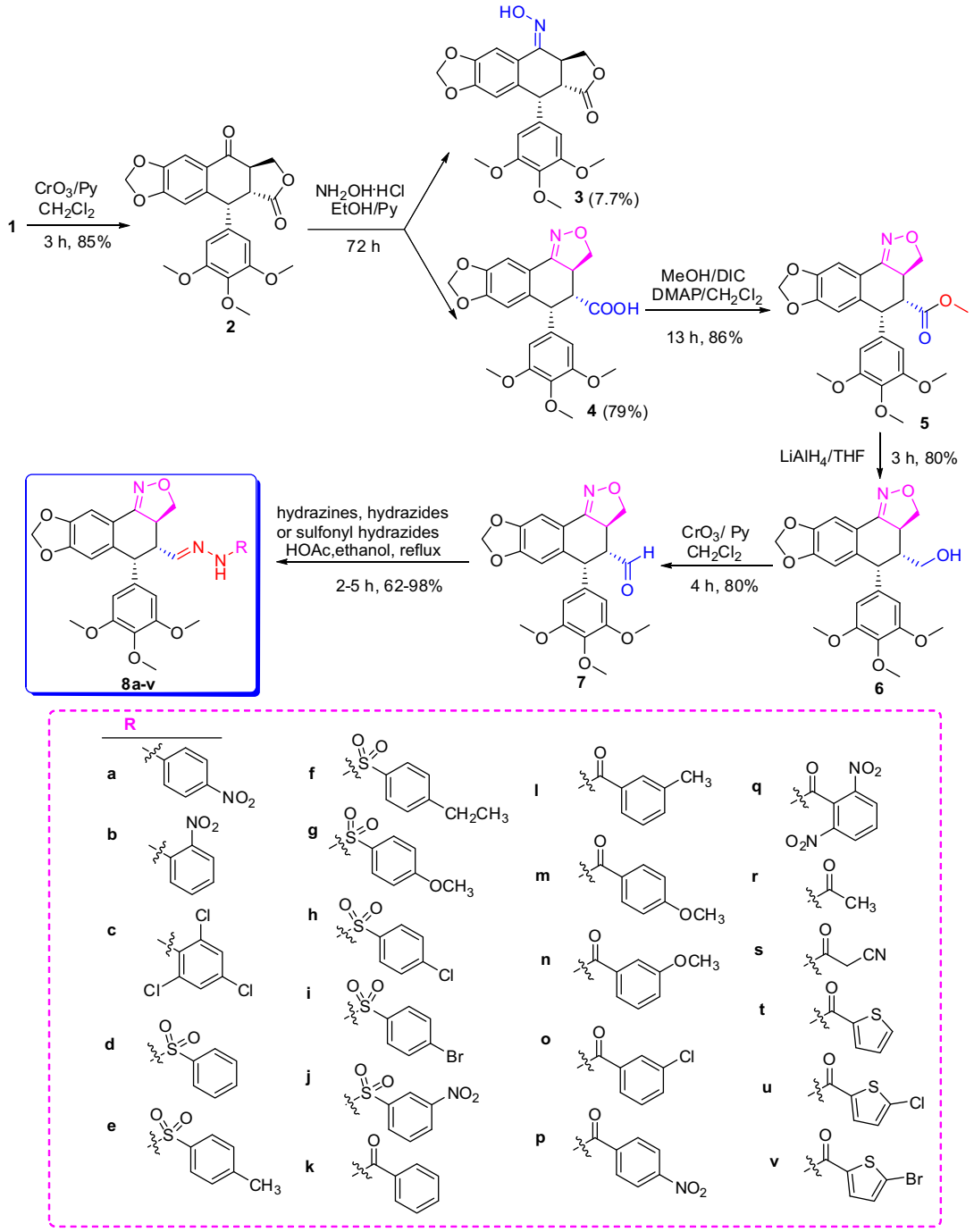

Scheme 1. The synthetic route for the preparation of **8a**–**v**.

As shown in Scheme 1, firstly oxidation of **1** in the presence of chromium trioxide (CrO_3) and pyridine afforded podophyllone (**2**). When **2** reacted with hydroxylamine hydrochloride, besides oxime of podophyllotoxone (**3**, 7.7% yield), isoxazolopodophyllic acid (**4**) was obtained as the major product in 79% yield. Then isoxazolopodophyllic acid-based ester (**5**) was obtained by the reaction of **4** with methanol in the presence of DIC and DMAP. Reduction of **5** in the presence of $LiAlH_4$ gave isoxazolopodophyllol (**6**), which was oxidized by CrO_3 and pyridine to afford **7**. Finally, **7** reacted with hydrazines, hydrazides or sulfonyl hydrazides to give podophyllotoxin-based hydrazone derivatives **8a**–**v**. Among them, the single-crystal structure of **8a** was illustrated in Figure 2. It clearly demonstrated that the substituents on the C=N double bond of **8a** adopted *E* configuration.[30]

The insecticidal activity of compounds **8a**–**v** against the pre-third-instar larvae of *M. separata* in vivo was tested by the leaf-dipping method at a concentration of 1 mg/mL.[31] Toosendanin, a commercial botanical insecticide isolated from *Melia azedarach*, was used as the positive control at 1 mg/mL. Leaves treated with acetone alone were used as a blank control group. As described in Table 1, the corresponding mortality rates of tested compounds after 35 days were generally higher than those after 10 and 20 days. Therefore, these compounds exhibited the delayed insecticidal activity. Moreover, the symptoms of the tested *M. separata* were also characterized by the same way as our previous reports.[24–29] Compared to toosendanin, especially compounds **8i**, **8j**, **8t**, and **8u** showed the more potent insecticidal activity with the final mortality rates greater than 60%. Interestingly,

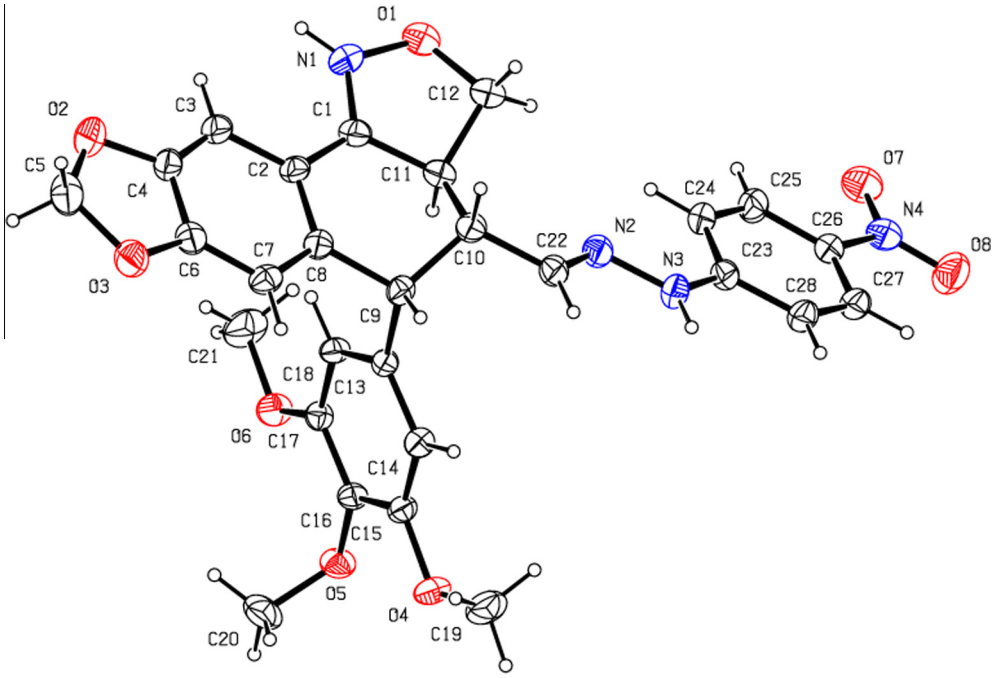

Figure 2. The X-ray crystal structure of **8a**.

Table 1
Insecticidal activity of **8a–v** against *M. separata* on leaves treated with a concentration of 1 mg/mL[a]

Compound	Corrected mortality rate (%)		
	10 days	20 days	35 days
1	6.7 ± 4.7	16.7 ± 12.5	40.0 ± 8.2
2	10.0 ± 8.2	20.0 ± 8.2	23.3 ± 4.7
3	6.7 ± 9.4	16.7 ± 4.7	36.7 ± 4.7
4	13.3 ± 4.7	26.7 ± 4.7	40.0 ± 0
5	16.7 ± 4.7	26.7 ± 4.7	36.7 ± 4.7
6	10.0 ± 0	16.7 ± 9.4	40.0 ± 8.2
7	10.0 ± 8.2	30.0 ± 0	40.0 ± 0
8a	3.3 ± 4.7	16.7 ± 9.4	30.0 ± 8.2
8b	3.3 ± 4.7	16.7 ± 4.7	23.3 ± 12.5
8c	3.3 ± 4.7	16.7 ± 12.5	30.0 ± 8.2
8d	16.7 ± 4.7	46.7 ± 4.7	53.3 ± 4.7
8e	3.3 ± 4.7	20.0 ± 8.2	23.3 ± 4.7
8f	16.7 ± 4.7	20.0 ± 8.2	50.0 ± 8.2
8g	23.3 ± 9.4	36.7 ± 4.7	56.7 ± 4.7
8h	10.0 ± 0	23.3 ± 12.5	36.7 ± 9.4
8i	20.0 ± 8.2	30.0 ± 8.2	63.3 ± 4.7
8j	20.0 ± 8.2	40.0 ± 8.2	60.0 ± 0
8k	3.3 ± 4.7	13.3 ± 4.7	46.7 ± 9.4
8l	13.3 ± 4.7	30.0 ± 8.2	53.3 ± 9.4
8m	16.7 ± 4.7	26.7 ± 9.4	33.3 ± 4.7
8n	3.3 ± 4.7	3.3 ± 4.7	36.7 ± 4.7
8o	16.7 ± 9.4	46.7 ± 4.7	53.3 ± 4.7
8p	3.3 ± 4.7	16.7 ± 4.7	43.3 ± 4.7
8q	0 ± 0	16.7 ± 4.7	30.0 ± 8.2
8r	6.7 ± 4.7	16.7 ± 9.4	46.7 ± 9.4
8s	3.3 ± 4.7	10.0 ± 8.2	30.0 ± 0
8t	6.7 ± 4.7	36.7 ± 4.7	60.0 ± 0
8u	26.7 ± 4.7	46.7 ± 4.7	63.3 ± 4.7
8v	20.0 ± 8.2	23.3 ± 9.4	36.7 ± 9.4
Toosendanin[b]	10.0 ± 0	23.3 ± 4.7	43.3 ± 4.7

[a] Values are means ± SD of three replicate.
[b] Toosendanin was used as a positive control at 1 mg/mL.

introduction of the bromine atom at the C-4 position on the arylsulfonyl group of **8d** led to the more promising compound **8i**, whereas introduction of the chlorine atom at the same position of **8d** resulted in the less active compound **8h** (53.3% for **8d** vs 63.3% for **8i** vs 36.7% for **8h**). Introduction of 2-thienylacyl hydrazones could result in more potent derivatives compared to toosendanin (e.g., 60% for **8t** and 63.3% for **6c**). It suggested that such hydrazides containing different heterocycles could be considered to introduce at the C-9 position of podophyllotoxin to prepare the hydrazones as insecticidal agents. However, introduction of acetyl or cyanoacetyl hydrazone led to less active derivatives (e.g., **8r** and **8s**).

In conclusion, a series of novel podophyllotoxin-based hydrazone derivatives were synthesized, and evaluated for their insecticidal activity against the pre-third-instar larvae of *M. separata* in vivo. Especially compounds **8i**, **8j**, **8t**, and **8u** exhibited the more potent insecticidal activity with the final mortality rates greater than 60%. It demonstrated that the hydrazides containing different heterocycles could be introduced at the C-9 position of podophyllotoxin to prepare the hydrazones as insecticidal agents in the future.

Acknowledgments

The present research was supported by National Natural Science Foundation of China (No. 31071737), and Special Funds of Central Colleges Basic Scientific Research Operating Expenses (YQ2013008).

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014.04.074.

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30. Crystallographic data (excluding structure factors) for the structure of **8a** have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC 944935. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44 (0)1223 336033 or e-mail: deposit@ccdc.cam.ac.uk].
31. *Biological assay:* The insecticidal activity of compounds **1**–**7**, and **8a**–**v** against the pre-third-instar larvae of *Mythimna separata* was assessed by leaf-dipping method. Toosendanin, a commercial insecticide isolated from *Melia azedarach*, was used as a positive control and supplied by Research & Development Center of Biorational Pesticide, Northwest A&F University, Shaanxi province, China. For each compound, 30 larvae (10 larvae per group) were used. Acetone solutions of all the above tested compounds, and toosendanin were prepared at the concentration of 1 mg/mL. Fresh wheat leaves were dipped into the corresponding solution for 3 s, then taken out, and dried in a room. Leaves treated with acetone alone were used as a blank control group. Several treated leaves were kept in each dish, where every 10 larvae were raised. If the treated leaves were consumed, additional treated leaves were added to the dish. After 48 h, untreated fresh leaves were added to all dishes until adult emergence. The experiment was carried out at 25 ± 2 °C and on 12/12 h (light/dark) photoperiod. The insecticidal activity of the tested compounds against the pre-third-instar larvae of *M. separata* was calculated by the following formula:
corrected mortality rate $(\%) = (T - C) \times 100/(100 - C)$
where T is the mortality rate in the treated group expressed as a percentage and C is the mortality rate in the untreated group expressed as a percentage.