Bioorganic & Medicinal Chemistry Letters 23 (2013) 5552–5557

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Natural-product-based insecticidal agents 14. Semisynthesis and insecticidal activity of new piperine-based hydrazone derivatives against *Mythimna separata* Walker in vivo

Huan Qu [a], Xiang Yu [a], Xiaoyan Zhi [a], Min Lv [b], Hui Xu [a,*]

[a] Laboratory of Pharmaceutical Design & Synthesis, College of Sciences, Northwest A&F University, Yangling 712100, PR China
[b] College of Plant Protection, Northwest A&F University, Yangling 712100, PR China

ARTICLE INFO

Article history:
Received 8 April 2013
Revised 30 July 2013
Accepted 12 August 2013
Available online 20 August 2013

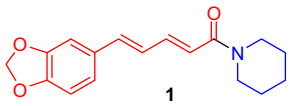
Keywords:
Piperine
Hydrazone
Insecticidal activity
Botanical insecticide
Structural modification
Mythimna separata Walker

ABSTRACT

In continuation of our program aimed at the discovery and development of natural-product-based insecticidal agents, twenty-six new piperine-based hydrazone derivatives were synthesized from piperine, an alkaloid isolated from *Piper nigrum* Linn. The single-crystal structures of **6c**, **6q** and **6w** were unambiguously confirmed by X-ray crystallography. Their insecticidal activity was evaluated against the pre-third-instar larvae of *Mythimna separata* Walker in vivo. Especially compounds **6b**, **6i** and **6r**, the final mortality rates of which, at the concentration of 1 mg/mL, were 62.1%, 65.5% and 65.5%, respectively, exhibited more pronounced insecticidal activity compared to toosendanin at 1 mg/mL, a commercial botanical insecticide isolated from *Melia azedarach*. It suggested that introduction of the substituents at the C-2 position on the phenyl ring of the hydrazone derivatives was important for their insecticidal activity.

Although synthetic chemical insecticides have played a significant role in modern agricultural pest management, 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 originated from plant secondary metabolites may lead to 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]

Piperine (**1**, Fig. 1), a simple alkaloid isolated from *Piper nigrum* Linn., has been found many medicinal activities such as monoamine oxidase (MAO) inhibitors,[6,7] liver CYP3A4 enzymatic inhibitors,[8] antiadipogenic activity,[9] antimicrobial activity,[10] anti-inflammatory activity,[11] positive allosteric GABA(A) receptor modulators,[12,13] Staphylococcus aureus NorA efflux pump inhibitor (EPI),[14] trypanocidal agents,[15,16] and cytochrome P450 (CYP) inhibitor.[17] Additionally, compound **1** and its related derivatives also exhibited the interesting insecticidal activity.[18,19] More recently, we have synthesized a series of fraxinellone-based hydrazone derivatives and found some compounds displayed the potent insecticidal activity.[20] In continuation of our program aimed at the discovery and development of natural-product-based insecticidal agents,[20–23] therefore, in the present Letter we prepared a series of piperine-based hydrazone derivatives as insecticidal agents (Scheme 1). Their insecticidal activity was evaluated against the pre-third-instar larvae of *Mythimna separata* Walker in vivo.



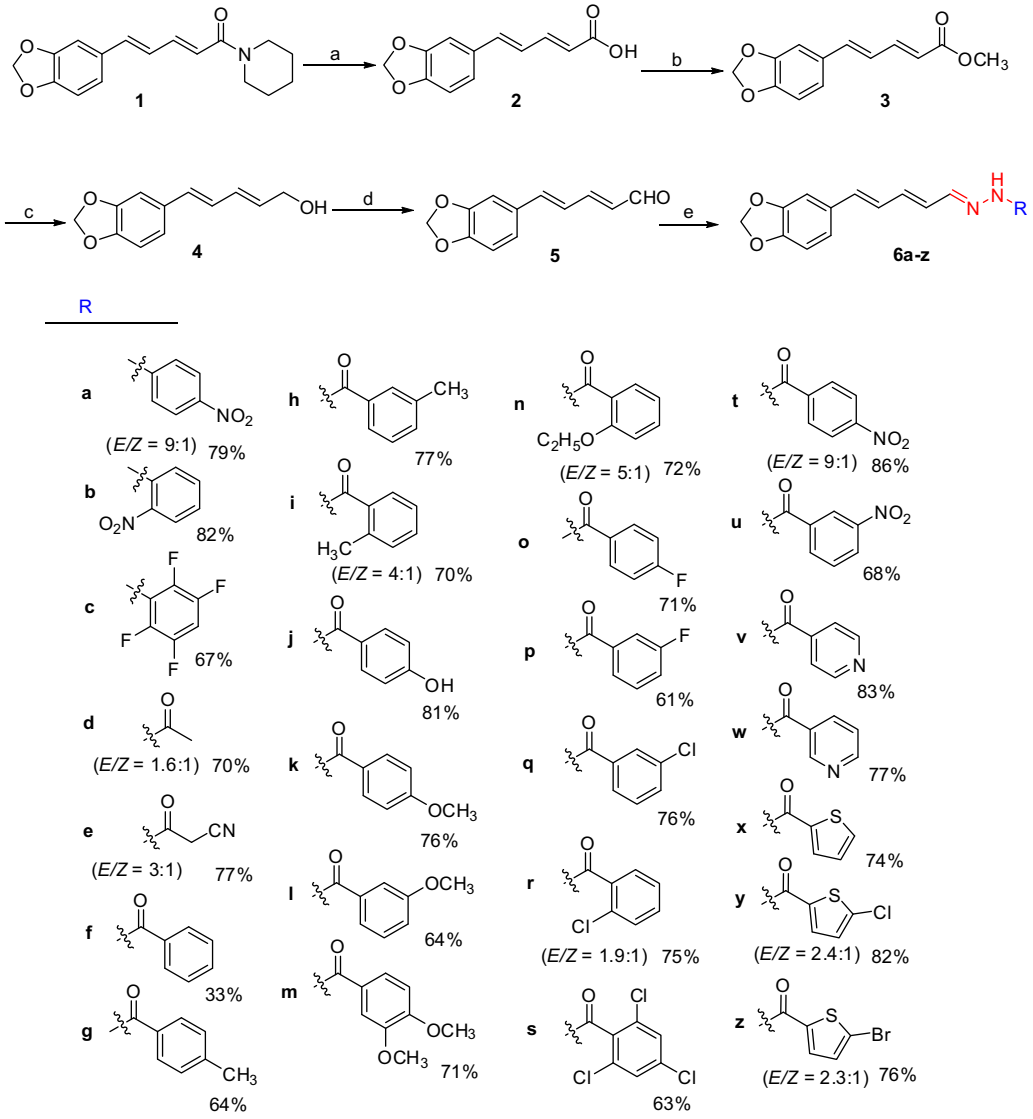
1

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

As shown in Scheme 1, the piperic acid (**2**) was obtained from **1** by the basic hydrolysis,[24] and methyl piperate (**3**) was smoothly prepared by reaction of **2** with methanol in the presence of concd sulfuric acid. Then reduction of **3** with $LiAlH_4$ and $AlCl_3$ gave (2*E*,4*E*)-5-(1,3-benzodioxol-5-yl)-2,4-pentadien-1-ol (**4**),[25] which was subsequently oxidized by MnO_2 to produce (2*E*,4*E*)-5-(1,3-benzodioxol-5-yl)-2,4-pentadienal (**5**).[16] Finally, compound **5** reacted with hydrazides or hydrazines to afford piperine-based hydrazone derivatives (**6a**–**z**), which were characterized by ^1H NMR, IR, HRMS and mp (see Supplementary data). Due to the steric hindrance, the substituents on the C≡N double bond of all

* Corresponding author. Tel./fax: +86 29 87091952.
E-mail address: orgxuhui@nwsuaf.edu.cn (H. Xu).

0960-894X/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmcl.2013.08.053



Scheme 1. Synthetic route for the preparation of **6a–z**. Reagents and conditions: (a) KOH, 95% ethanol, reflux, 16 h, 93%; (b) methanol, H_2SO_4, reflux, 16 h, 91%; (c) $LiAlH_4$/$AlCl_3$, THF, 0 °C, 4 h, 58%; (d) MnO_2, THF, reflux, 4 h, 75%; (e) hydrazides or hydrazines, ethanol, HOAc, reflux, 1–3 h, 33–86%.

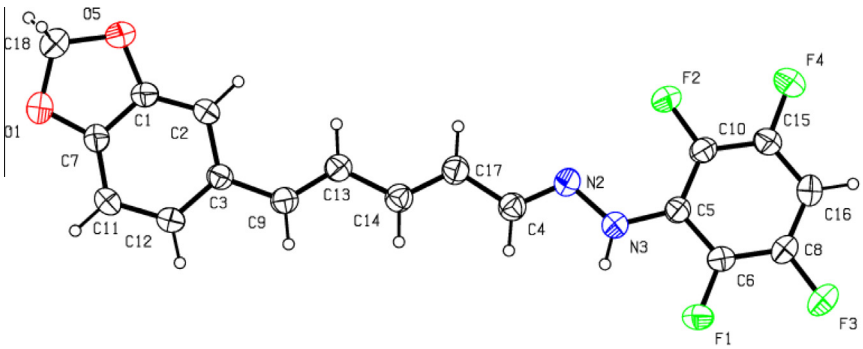


Figure 2. The X-ray crystal structure of **6c**.

compounds (except **6a**, **6d**, **6e**, **6i**, **6n**, **6r**, **6t**, **6y** and **6z**) adopted *E* configuration. The ratios of *E*/*Z* of **6a**, **6d**, **6e**, **6i**, **6n**, **6r**, **6t**, **6y** and **6z** were 9/1, 1.6/1, 3/1, 4/1, 5/1, 1.9/1, 9/1, 2.4/1, and 2.3/1, respectively.[26] To obtain the precise three-dimensional structural information of **6a–z**, the single-crystal structures of **6c**, **6q** and **6w** were well determined by X-ray crystallography (Figs. 2–4).[27] The substituents on the C≡N double bond of **6c**, **6q** and **6w** all adopted *E* configuration, which is found during crystal packing.

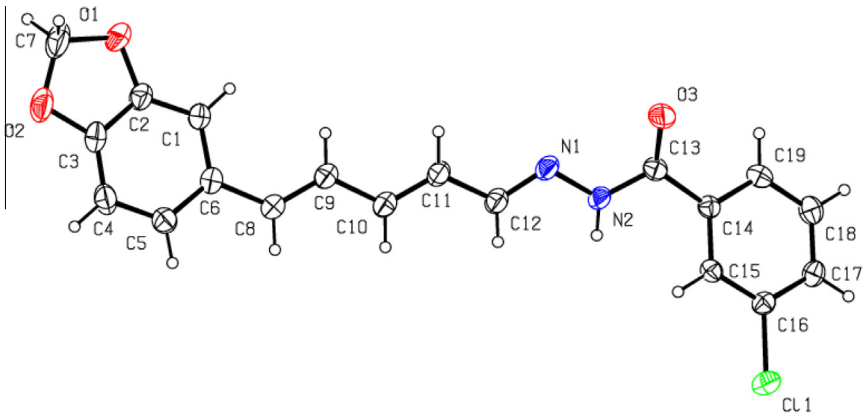


Figure 3. The X-ray crystal structure of **6q**.

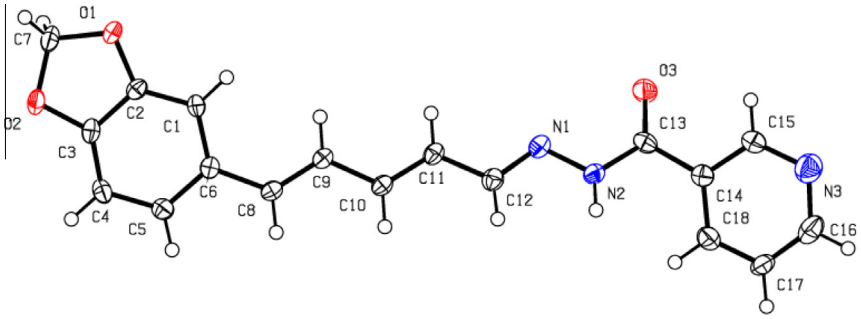


Figure 4. The X-ray crystal structure of **6w**.

The insecticidal activity of **6a–z** was tested against the pre-third-instar larvae of *Mythimna separata* Walker in vivo by the leaf-dipping method at the concentration of 1 mg/mL.[23,28] Toosendanin, a commercial botanical insecticide isolated from *Melia azedarach*, was used as the positive control at 1 mg/mL, and leaves treated with acetone alone were used as a blank control group. Analysis of variance (ANOVA) was followed by Duncan's post test, which was conducted using SPSS 20 for Windows 7.

As described in Table 1, the corresponding mortality rates of **6a–z** after 34 days were usually higher than those after 10 and 20 days. Therefore, these piperine-based hydrazone derivatives, in a time-dependent manner, different from those conventional neurotoxic insecticides such as organophosphates, carbamates, and pyrethroids, exhibited delayed insecticidal activity. Meanwhile, the symptoms of the tested *M. separata* were also characterized by the same way as our previous reports.[20–23] Due to feeding too much treated leaves during the first 48 h, some larvae died slowly as evidenced from slim and wrinkled bodies during the larval period (Fig. 5). In the meantime, many larvae of the treated groups moulted to malformed pupae, and died during the stage of pupation (Fig. 6). Malformed moths with imperfect wings were also appeared in the treated groups (Fig. 7). According to the symptoms of the tested *M. separata*, the above derivatives possibly displayed the anti-molting hormone effect.

As shown in Table 1, if the amide group of **1** was substituted by a carboxyl, ester, alcohol, or aldehyde moiety, the corresponding derivatives such as **2**, **3**, **4**, and **5** did not exhibit the more potent insecticidal activity compared to their precursor piperine. Interestingly, when **5** reacted with hydrazides or hydrazines to afford piperine-based hydrazone derivatives **6a–z**, some compounds such as **6a**, **6b**, **6i**, **6o** and **6r** showed the more potent insecticidal activity than their precursor piperine. Especially compounds **6b**, **6i** and **6r** displayed the most promising and pronounced insecticidal activity compared to toosendanin. For example, the final mortality rates of **6b**, **6i** and **6r** were 62.1%, 65.5% and 65.5%, respectively;

Table 1
Insecticidal activity of **6a–z** against *M. separata* on leaves treated with a concentration of 1 mg/mL[a]

Compounds	Corrected mortality rate (%)		
	10 days	20 days	34 days
1	23.3 efg[b]	37.9 fgh	48.3 fgh
2	6.7 ab	24.1 bcd	37.9 cde
3	10.0 abc	34.5 efg	51.7 ghi
4	6.7 ab	20.7 abc	34.5 bcd
5	20.0 def	27.6 cde	48.3 fgh
6a	10.0 abc	41.4 gh	55.2 hij
6b	10.0 abc	37.9 fgh	62.1 jk
6c	13.3 bcd	27.6 cde	48.3 fgh
6d	16.7 cde	20.7 abc	41.4 def
6e	23.3 efg	27.6 cde	37.9 cde
6f	3.3 a	20.7 abc	34.5 bcd
6g	20.0 def	24.1 bcd	41.4 def
6h	16.7 cde	31.0 def	44.8 efg
6i	23.3 efg	41.4 gh	65.5k
6j	10.0 abc	27.6 cde	34.5 bcd
6k	10.0 abc	20.7 abc	34.5 bcd
6l	13.3 bcd	37.9 fgh	44.8 efg
6m	10.0 abc	27.6 cde	48.3 fgh
6n	3.3 a	17.2 ab	34.5 bcd
6o	30.0g	41.4 gh	58.6 ijk
6p	13.3 bcd	24.1 bcd	44.8 efg
6q	6.7 ab	20.7 abc	34.5 bcd
6r	30.0g	44.8h	65.5k
6s	10.0 abc	24.1 bcd	48.3 fgh
6t	3.3 a	31.0 def	44.8 efg
6u	6.7 ab	20.7 abc	37.9 cde
6v	6.7 ab	17.2 ab	44.8 efg
6w	26.7fg	31.0 def	44.8 efg
6x	6.7ab	24.1bcd	31.0abc
6y	3.3a	17.2ab	24.1a
6z	6.7 ab	13.8 a	27.6 ab
Toosendanin	20.0 def	41.4 gh	51.7 ghi

[a] Values are means ± SD of three replicate.
[b] Multiple range test using Duncan's test (p <0.05). The same letters denote treatments not significantly different from each other.

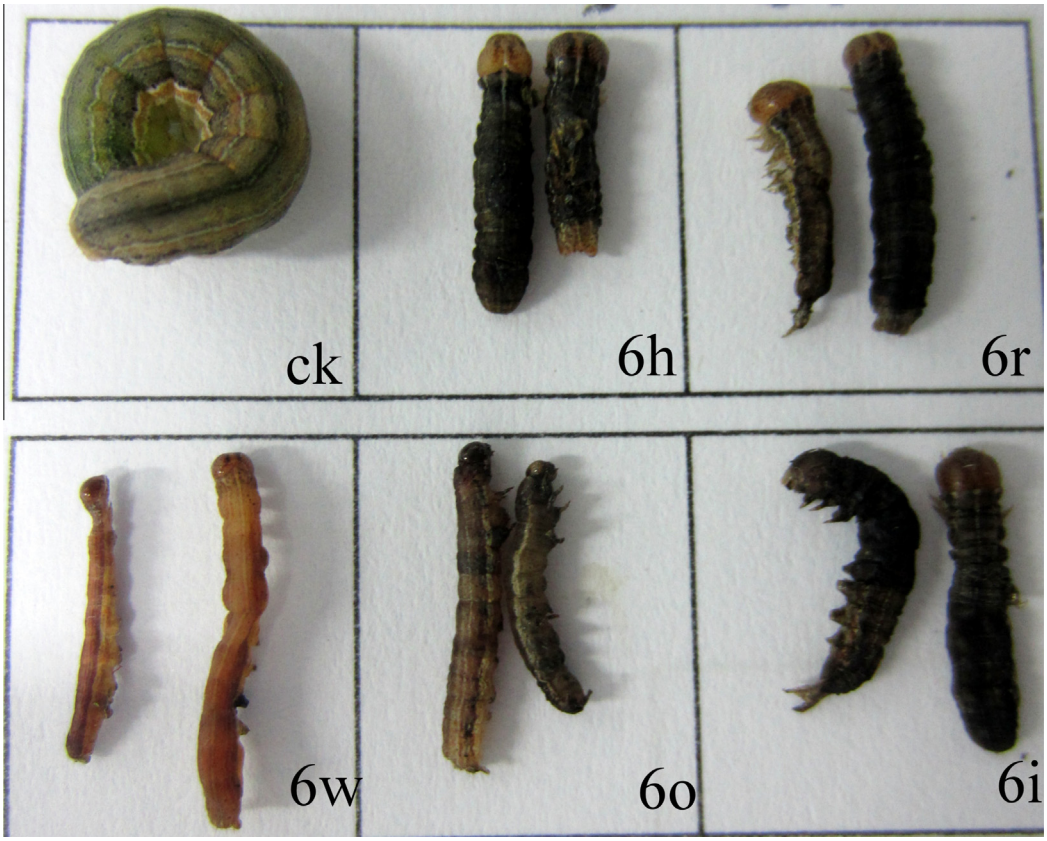


Figure 5. The representative abnormal larvae pictures of **6h**, **6i**, **6o**, **6r**, and **6w** during the larval period (**CK**: blank control group).

Figure 6. The representative malformed pupae pictures of **6a**, **6i**, **6k**, **6r**, and **6y** during the pupation period (**CK**: blank control group).

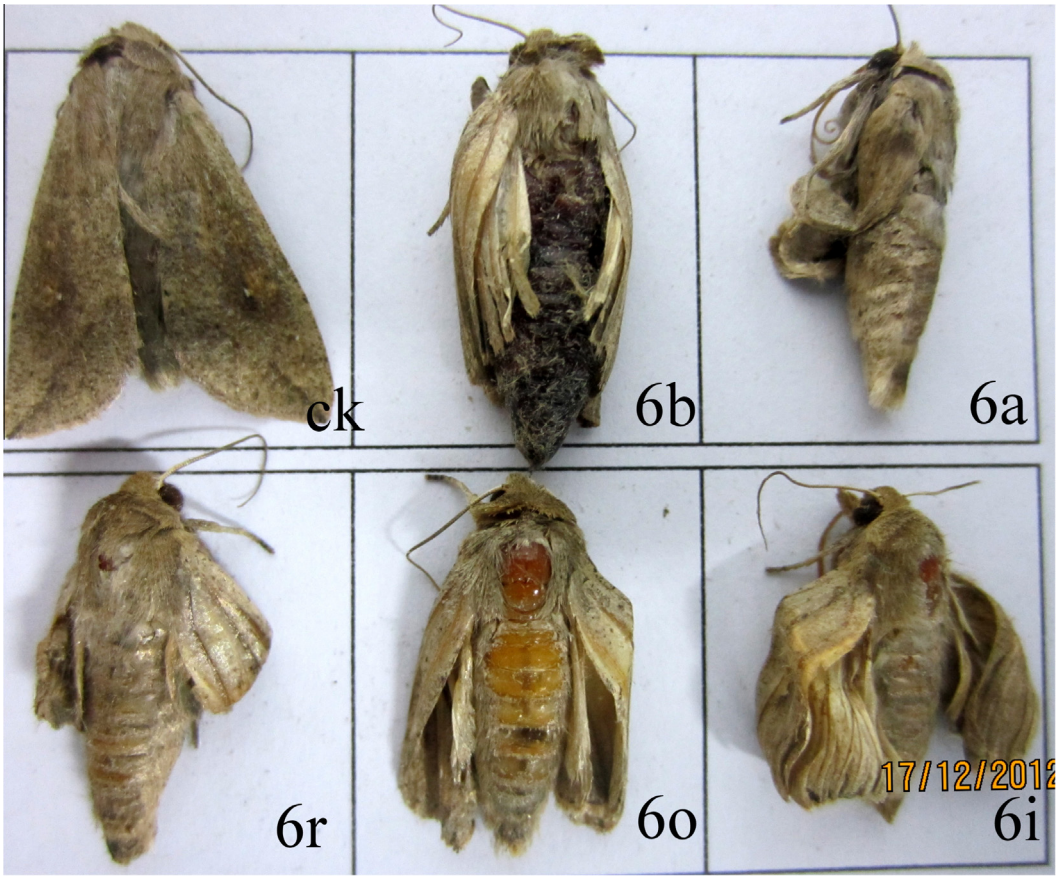


Figure 7. The representative malformed moth pictures of **6a**, **6b**, **6i**, **6o**, and **6r** during the emergence period (**CK**: blank control group).

whereas the final mortality rates of toosendanin was 51.7%. Introduction of the substituents at the C-2 position on the phenyl ring of the hydrazone derivatives was important to their insecticidal activity. For example, the final mortality rates of **6g** (containing 4-methyl on the phenyl ring) and **6h** (containing 3-methyl on the phenyl ring) were 41.4% and 44.8%, respectively; whereas the final mortality rate of **6i** (containing 2-methyl on the phenyl ring) was 65.5%. Similarly, the final mortality rates of **6q** (containing 3-Cl on the phenyl ring) and **6s** (containing 2,4,6-trichloro on the phenyl ring) were 34.5% and 48.3%, respectively; whereas the final mortality rate of **6r** (containing 2-Cl on the phenyl ring) was 65.5%. However, introduction of the substituents *R* as the alkylcarbonyl (e.g., **6d** and **6e**) or the heterocyclylcarbonyl groups (e.g., **6v**–**z**) could not lead to the more potent compounds compared to their precursor piperine.

In summary, a series of new piperine-based hydrazone derivatives (**6a**–**z**) were prepared by structural modification of piperine. Their insecticidal activity was evaluated against the pre-third-instar larvae of *M. separata* in vivo. Among them, especially **6b**, **6i** and **6r** exhibited the more pronounced insecticidal activity compared to toosendanin. It suggested that introduction of the substituents at the C-2 position on the phenyl ring of the hydrazone derivatives was necessary for their insecticidal activity.

Acknowledgments

This work was supported by National Natural Science Foundation of China (No. 31171896), and the Special Funds of Central Colleges Basic Scientific Research Operating Expenses (YQ2013008) to Dr. Xu.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.08.053.

References and notes

1. Pavela, R. *Pest Technol.* **2007**, *1*, 47.
2. Heckel, D. G. *Science* **2012**, *337*, 1612.
3. Rios-Diez, J. D.; Saldamando-Benjumea, C. I. *J. Econ. Entomol.* **2011**, *104*, 1698.
4. Isman, M. B. *Annu. Rev. Entomol.* **2006**, *51*, 45.
5. Dayan, F. E.; Cantrell, C. L.; Duke, S. O. *Bioorg. Med. Chem.* **2009**, *17*, 4022.
6. Mu, L. H.; Wang, B.; Ren, H. Y.; Liu, P.; Guo, D. H.; Wang, F. M.; Bai, L.; Guo, Y. S. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 3343.
7. Rahman, T.; Rahmatullah, M. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 537.
8. Makhov, P.; Golovine, K.; Canter, D.; Kutikov, A.; Simhan, J.; Corlew, M. M.; Uzzo, R. G.; Kolenko, V. M. *Prostate* **2012**, *72*, 661.
9. Park, U. H.; Jeong, H. S.; Jo, E. Y.; Park, T.; Yoon, S. K.; Kim, E. J.; Jeong, J. C.; Um, S. J. *J. Agric. Food Chem.* **2012**, *60*, 3853.
10. Mirza, Z. M.; Kumar, A.; Kalia, N. P.; Zargar, A.; Khan, I. A. *J. Med. Microbiol.* **2011**, *60*, 1472.
11. Bae, G. S.; Kim, M. S.; Jung, W. S.; Seo, S. W.; Yun, S. W.; Kim, S. G.; Park, R. K.; Kim, E. C.; Song, H. J.; Park, S. J. *Eur. J. Pharmacol.* **2010**, *642*, 154.
12. Zaugg, J.; Baburin, I.; Strommer, B.; Kim, H. J.; Hering, S.; Hamburger, M. *J. Nat. Prod.* **2010**, *73*, 185.
13. Nargotra, A.; Sharma, S.; Koul, J. L.; Sangwan, P. L.; Khan, I. A.; Kumar, A.; Taneja, S. C.; Koul, S. *Eur. J. Med. Chem.* **2009**, *44*, 4128.
14. Sangwan, P. L.; Koul, J. L.; Koul, S.; Reddy, M. V.; Thota, N.; Khan, I. A.; Kumar, A.; Kalia, N. P.; Qazi, G. N. *Bioorg. Med. Chem.* **2008**, *16*, 9847.
15. Ferreira, W. D.; Freire-De-Lima, L.; Saraiva, V. B.; Alisson-Silva, F.; Mendonca-Previato, L.; Previato, J. O.; Echevarria, A.; Freire de Lima, M. E. *Bioorg. Med. Chem.* **2008**, *16*, 2984.
16. Ribeiro, T. S.; Freire-de-Lima, L.; Previato, J. O.; Mendonca-Previato, L.; Heise, N.; De Lima; Freire de Lima, M. E. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3555.
17. Koul, S.; Koul, J. L.; Taneja, S. C.; Dhar, K. L.; Jamwal, D. S.; Singh, K.; Reen, R. K.; Singh, J. *Bioorg. Med. Chem.* **2000**, *8*, 251.

18. de Paula, V. F.; de A Barbosa, L. C.; Demuner, A. J.; Pilo-Veloso, D.; Picanco, M. C. *Pest Manag. Sci.* **2000**, *56*, 168.
19. Tavares, W. S.; Cruz, I.; Petacci, F.; Freitas, S. S.; Serrao, J. E.; Zanuncio, J. C. *J. Med. Plant Res.* **2011**, *5*, 5301.
20. Guo, Y.; Yan, Y. Y.; Yang, C.; Yu, X.; Zhi, X. Y.; Xu, H. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 5384.
21. Xu, H.; Zhang, J. L. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 1974.
22. Xu, H.; Wang, Q. T.; Guo, Y. *Chem. Eur. J.* **2011**, *17*, 8299.
23. Wang, Y.; Shao, Y.; Wang, Y.; Fan, L.; Yu, X.; Zhi, X.; Yang, C.; Qu, H.; Yao, X.; Xu, H. *J. Agric. Food Chem.* **2012**, *60*, 8435.
24. Correa, E. A.; Hogestatt, E. D.; Sterner, O.; Echeverri, F.; Zygmunt, P. M. *Bioorg. Med. Chem.* **2010**, *18*, 3299.
25. Li, Y.; Zhang, Y.; Huang, Z.; Cao, X.; Gao, K. *Can. J. Chem.* **2004**, *82*, 622.
26. The ratios of *E*/*Z* of **6a**, **6d**, **6e**, **6i**, **6n**, **6r**, **6t**, **6y** and **6z** were determined by the ^{1}H NMR spectra (see Supplementary data).
27. Crystallographic data (excluding structure factors) for the structures of **6c**, **6q** and **6w** in this Letter have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC 932634, 932635, and 932633, respectively. 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].
28. *Bioassay:* The insecticidal activity of **6a**–**z** against the pre-third-instar larvae of *Mythimna separata* Walker was assessed by leaf-dipping method. For each compound, 30 larvae (10 larvae per group) were used. Acetone solutions of **6a**–**z** and toosendanin (used as a positive control) 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 control group. Several treated leaves were kept in each dish, where every 10 larvae were raised. If the treated leaves were consumed, the corresponding ones were added to the dish. After 48 h, untreated fresh leaves were added to the all dish until the adult emergence. The experiment was carried out at 25 ± 2 °C and relative humidity (RH) 65–80%, and on 12 h/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 (%) = $(T-C) \times 100/(1-C)$
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.