Bioorganic & Medicinal Chemistry Letters 20 (2010) 5009–5012

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Natural products-based insecticidal agents 7. Semisynthesis and insecticidal activity of novel 4α-alkyloxy-2-chloropodophyllotoxin derivatives against *Mythimna separata* Walker in vivo

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ARTICLE INFO

Article history:
Received 5 May 2010
Revised 19 June 2010
Accepted 13 July 2010
Available online 16 July 2010

Keywords:
Alkyloxy
Podophyllotoxin
2-Chloropodophyllotoxin
Semisynthesis
Insecticidal activity

ABSTRACT

In continuation of our program aimed at the discovery and development of natural products-based insecticidal agents, 16 novel 4α-alkyloxy-2-chloropodophyllotoxin derivatives were semisynthesized from podophyllotoxin, and preliminarily evaluated for their insecticidal activity against the pre-third-instar larvae of *Mythimna separata* Walker in vivo. Among all the tested derivatives, especially compounds **4b**, **4e**, **4g**, and **4p** exhibited more promising and pronounced insecticidal activity than toosendanin, a commercial insecticide derived from *Melia azedarach*. Generally, it was obviously demonstrated that the length of straight-chain or branched-chain alkyloxy, and heteroatom-containing cycloalkyloxy at the C-4 position of 2-chloropodophyllotoxin were very important for the insecticidal activity.

The routine use of a wide variety of synthetic insecticides in agriculture has now become an accepted practice, however, the application of those chemicals over the years has led to the development of resistance in insect pest populations and environmental problems. Meanwhile, plant secondary metabolites result from the interaction between plants and environment (life and non-life) during the long period of evolution in plants. Consequently, the discovery and development of new insecticidal compounds from plant secondary metabolites, followed by using them as the lead-compounds for further modification has recently been one of the important ways for the research and development of new pesticides. Podophyllotoxin (**1**, Fig. 1), a naturally occurring aryltetralin lignan, besides its use as the lead-compound for the preparation of potent anticancer drugs,[1] also exhibited the interesting insecticidal activity.[2–4]

More recently, the insecticidal activity of 4β-benzenesulfonamides of podophyllotoxin,[5] 4′-aromatic esters/substituted benzenesulfonates of 4-deoxypodophyllotoxin,[6,7] and 4α-acyloxy-2-

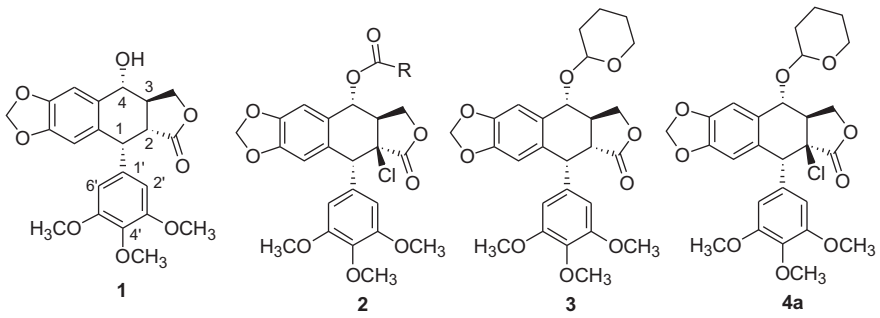

Figure 1. Chemical structures of podophyllotoxin (**1**), 4α-acyloxy-2-chloropodophyllotoxins (**2**), 4-*O*-tetrahydropyranylpodophyllotoxin (**3**), and 2-chloro-4-*O*-tetrahydropyranylpodophyllotoxin (**4a**).

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doi:10.1016/j.bmcl.2010.07.050

chloropodophyllotoxins (**2**, Fig. 1)[8] has been studied in our research group, and some derivatives have showed the potent insecticidal activity. During investigation of structure–insecticidal activity relationships of **2**, interestingly, 2-chloro-4-*O*-tetrahydropyranylpodophyllotoxin (**4a**, Fig. 1), an intermediate of **2**, exhibited more potent insecticidal activity than 4-*O*-tetrahydropyranylpodophyllotoxin (**3**, Fig. 1) and toosendanin, a commercial insecticide derived from *Melia azedarach*.[8] That is, introduction of chlorine atom at the 2β position of **3** led to the more potent compound **4a**. This encouraging result, therefore, prompted us in present Letter to further study other 4-alkyloxy derivatives of 2-chloropodophyllotoxin as insecticidal agents.

Sixteen novel 4α-alkyloxy-2-chloropodophyllotoxin derivatives (**4b–q**) were synthesized from podophyllotoxin (**1**) as outlined in Scheme 1. The 4-OH group of **1** was firstly protected by a tetrahydropyranyl (THP) group in the presence of phosphorus oxychloride ($POCl_3$) and dihydropyran (DHP) at room temperature to give 4-*O*-tetrahydropyranylpodophyllotoxin (**3**) in a 92% yield.[9] 2-Chloro-4-*O*-tetrahydropyranylpodophyllotoxin (**4a**) was then prepared by treatment of **3** with lithium diisopropylamide (LDA) at −78 °C in dry THF, followed by the stereoselective reaction with hexachloroethane. Subsequently, hydrolysis of the THP group of **4** afforded 2-chloropodophyllotoxin (**5**).[10] Finally, 16 novel 4α-alkyloxy-2-chloropodophyllotoxin derivatives (**4b–q**) were obtained in 13–78% yields by reaction of **5** with the corresponding alcohols in the presence of $BF_3{\cdot}Et_2O$. The structures of the target compounds were well characterized by ^1H NMR, HRMS, mp, and IR (see Supplementary data).

The assignment of configuration of C-4 position was based on $J_{3,4}$ coupling constants. The C-4β-substituted compounds have a $J_{3,4} \approx 4.0$ Hz due to a *cis* relationship between H-3 and H-4. If $J_{3,4} \geqq 10.0$ Hz, it indicates that H-3 and H-4 is *trans* relationship, and the substituent on the C-4 position of podophyllotoxin is α configuration.[11] For example, the $J_{3,4}$ value of H-4 of **4d** was 9.2 Hz, therefore, the hydroxylethoxy group on the C-4 position of **4d** was α configuration.

In order to obtain precise three-dimensional structural information and absolute configuration of **4b–q**, the single-crystal structure of **4h** was determined by X-ray crystallography as illustrated in Figure 2.[12] It was clearly demonstrated that the 2-chloro and the 4-*n*-butoxy groups of **4h** adopted the β and α configuration, respectively.

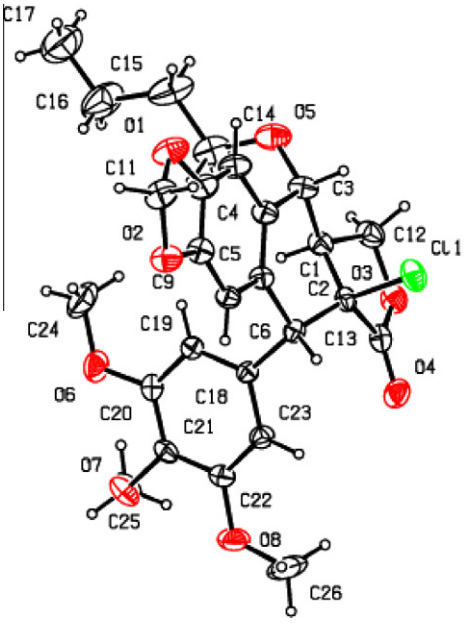
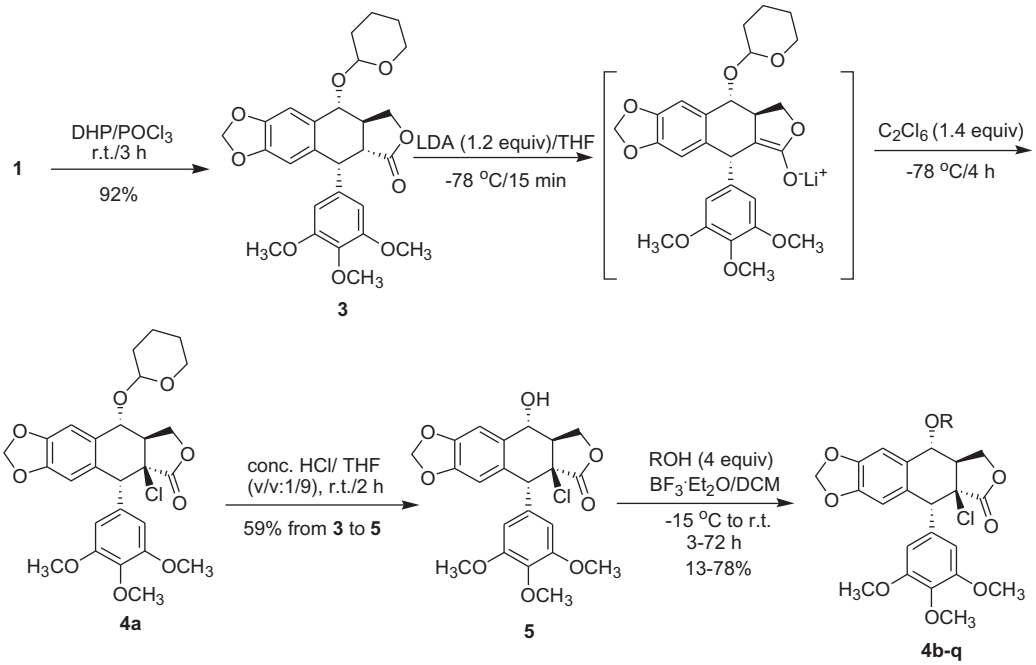


Figure 2. The X-ray crystallography of compound **4h**.

The insecticidal activity of **4b–q** against the pre-third-instar larvae of *Mythimna separata* Walker in vivo was screened by the leaf-dipping method at the concentration of 1 mg/mL. Compound **4a**, and toosendanin, a commercial insecticide derived from *M. azedarach*, were used as positive controls.[8]

The corrected mortality rates of *M. separata* caused by **4b–q** with the advance of time were shown in Figure 3. The corresponding mortality rates after 35 d were far higher than those after 10 d and 20 d. That is, these compounds, different from those conven-



Scheme 1. The synthetic route of 4α-alkyloxy-2-chloropodophyllotoxins (**4b–q**).

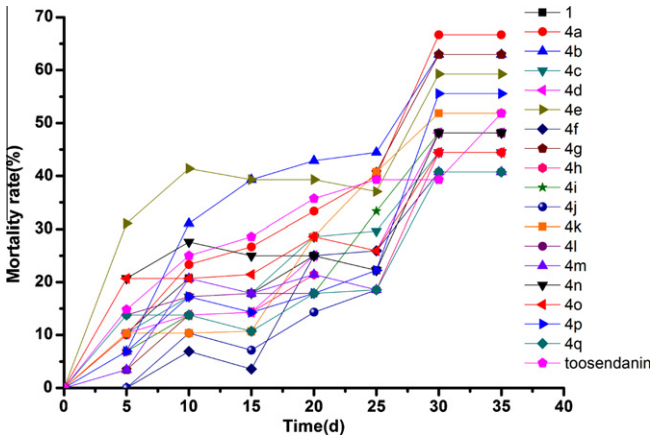
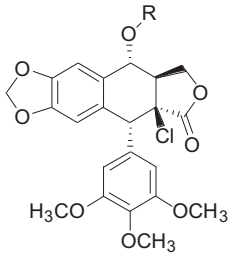


Figure 3. The corrected mortality rates of *M. separata* caused by **4b–q** with the increase of time.

tional neurotoxic insecticides, such as organophosphates, carbamates, and pyrethroids, showed delayed insecticidal activity.[5–8] For example, the corrected mortality rate of **4g** against *M. separata* after 10 d was only 13.8%, after 20 d the corresponding mortality rate was increased to 28.6%, but after 35 d the corresponding mortality rate was rapidly increased to 63%, which was more than four times of the mortality rate after 10 d (Table 1). Meanwhile, the symptoms of the tested *M. separata* were also characterized by the same way as our previous reports.[5–8] For example, the pupation of the larvae and the adult emergence of *M. separata* were inhibited by these compounds, therefore, the stage from the larvae to adulthood of *M. separata* was prolonged as compared with the control group. Moreover, many larvae of the treated groups molted to abnormal pupae, which could not reach adulthood and died during the stage of pupation because they were not able to remove their pupal skin.

Through a comparative study on the relationship between the chemical structures of **4b–q** and the insecticidal activity as outlined in Table 1, some interesting results were found as follows: (1) Generally, when methoxy, methoxyethoxy, isopropyloxy, and benzyloxy moieties were introduced at the C-4 position of 2-chloropodophyllotoxin, respectively, the corresponding compounds **4b**, **4e**, **4g**, and **4p**, the activity of which could comparable to that of some 4α-acyloxy derivatives of 2β-chloropodophyllotoxin,[8] displayed more promising and pronounced insecticidal activity than toosendanin. (2) Interestingly, the length of the side chain (for *n*-alkyloxy and isopropyloxy series) at the C-4 position of 2-chloropodophyllotoxin was very essential for its insecticidal activity. In general, as the length of the side chain at the C-4 position increased, the corresponding activity was reduced (**4b** vs **4c**, **4f**, **4h**, **4k**, and **4m**; **4g** vs **4i**, **4j**, and **4l**). For example, to isopropyloxy series, the final mortality rates of **4i**, **4j**, and **4l** were 48.2%, 40.7%, and 48.2%, respectively, while the final mortality rate of **4g** was 63%. (3) Introduction of methoxyethoxy moiety at the C-4 position of 2-chloropodophyllotoxin produced the more potent compound than that bearing hydroxylethoxy one at the C-4 position (**4e** 59.3% vs **4d** 48.2%). It implied that the free hydroxyl group on the side chain was not necessary for the insecticidal activity. (4) No significant differences in the activity were observed between **4n** and **4o**, which contained cyclopentyloxy and cyclohexyloxy groups at the C-4 position of 2-chloropodophyllotoxin, respectively. However, introduction of oxygen atom at the cyclohexyl ring of **4o** led to the more potent compound **4a** (**4a** 66.7% vs **4o** 44.4%). This interesting result will encourage us to further investigate the heteroatom-containing 4α-cyclopentyloxy/cyclohexyloxy-2-chloropodophyllotoxin derivatives as the insecticidal agents in future. (5)

Table 1
Insecticidal activity of novel 4α-alkyloxy-2-chloropodophyllotoxins (**4b–q**) against *M. separata* in vivo



4a-q

Compounds	R	Corrected mortality rate (%)		
		10 d	20 d	35 d
4a	Tetrahydropyranyl	23.3 (±12.5)	33.3 (±4.7)	66.7 (±4.7)
4b	Me	31.0 (±9.4)	42.9 (±4.7)	63.0 (±4.7)
4c	Et	17.2 (±0)	28.6 (±9.4)	44.4 (±0)
4d	CH_2CH_2OH	13.8 (±4.7)	25.0 (±0)	48.2 (±9.4)
4e	CH_2CH_2OMe	41.4 (±4.7)	39.3 (±4.7)	59.3 (±4.7)
4f	*n*-Pr	6.9 (±8.2)	25.0 (±8.2)	40.7 (±4.7)
4g	*i*-Pr	13.8 (±4.7)	28.6 (±4.7)	63.0 (±4.7)
4h	*n*-Bu	17.2 (±0)	21.4 (±4.7)	44.4 (±0)
4i	MeCHEt	13.8 (±12.5)	17.9 (±9.4)	48.2 (±4.7)
4j	$(CH_2)_2CH(Me)_2$	10.3 (±9.4)	14.3 (±8.2)	40.7 (±4.7)
4k	*n*-Octyl	10.3 (±12.5)	28.6 (±4.7)	51.9 (±9.4)
4l	$(CH_2)_5CH(Me)_2$	17.2 (±16.3)	17.9 (±4.7)	48.2 (±4.7)
4m	$n\text{-}C_{18}H_{37}$	20.7 (±9.4)	21.4 (±4.7)	40.7 (±4.7)
4n	Cyclopentyl	27.6 (±8.2)	25.0 (±8.2)	48.2 (±9.4)
4o	Cyclohexyl	20.7 (±17)	28.6 (±9.4)	44.4 (±8.2)
4p	CH_2Ph	17.2 (±8.2)	17.9 (±4.7)	55.6 (±8.2)
4q	$CH_2Ph(p\text{-}NO_2)$	13.8 (±12.5)	17.9 (±9.4)	40.7 (±12.5)
1	—	20.7 (±12.5)	25.0 (±8.2)	48.2 (±9.4)
3	—	10.7 (±9.4)	14.3 (±14.1)	51.9 (±12.5)
Toosendanin	—	25.0 (±0)	35.7 (±8.2)	51.9 (±9.4)

Introduction of benzyloxy moiety at the C-4 position of 2-chloropodophyllotoxin gave the more potent compound than that bearing (*p*-nitro)benzyloxy one at the C-4 position (**4p** 55.6% vs **4q** 40.7%).

In conclusion, 16 novel 4α-alkyloxy-2-chloropodophyllotoxin derivatives were semisynthesized from podophyllotoxin, and preliminarily evaluated for their insecticidal activity against the pre-third-instar larvae of *M. separata* in vivo. Among all the tested derivatives, especially compounds **4b**, **4e**, **4g**, and **4p** exhibited more promising and pronounced insecticidal activity than toosendanin. Generally, it was clearly confirmed that the length of straight-chain or branched-chain alkyloxy, and heteroatom-containing cycloalkyloxy at the C-4 position of 2-chloropodophyllotoxin were very important for the insecticidal activity.

Acknowledgments

This work was financially supported by the Program for New Century Excellent University Talents, State Education Ministry of China (NCET-06-0868), the Research Fund for the Doctoral Program of Higher Education of China (No. 20070712025), and the Fok Ying Tong Education Foundation for Young Talents (No. 121032).

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.07.050.

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12. Crystallographic data (excluding structure factors) for the structure of **4h** in this paper has been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC 774544. 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].