Bioorg. Med. Chem. Lett. 43 (2021) 128104

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Insecticidal activity of twin compounds from podophyllotoxin and cytisine

Yuanyuan Zhang, Min Lv *, Hui Xu *

College of Plant Protection/Chemistry and Pharmacy, Northwest A&F University, Yangling 712100, Shaanxi Province, PR China

ARTICLE INFO

Keywords:
Twin insecticides
Botanical bioactive product
Insecticidal activity
Podophyllum hexandrum
Thermopsis lanceolata

ABSTRACT

To explore natural-product-based insecticide candidates, and high value-added application of natural plants in agriculture, a series of twin compounds were prepared from two natural products podophyllotoxin and cytisine, which are isolated from the plants *Podophyllum hexandrum* and *Thermopsis lanceolata*, respectively. Compounds **IIa** (X = Cl, Y = R^1 = R^2 = H), **IIIc** (X = Y = R^1 = R^2 = Cl) and **IVd** (X = R^1 = R^2 = Br, Y = H) exhibited >2-fold potent insecticidal activity of podophyllotoxin against armyworm with FMRs greater than 60%. SARs were also observed. It is noteworthy that the idea of twin insecticides was addressed for the first time. We hope this idea will be conducive to design new twin insecticidal agents, and lay the foundation for future high value-added application of the plants *P. hexandrum* and *T. lanceolata* as potentially botanical pesticides in agriculture.

Podophyllotoxin (**1**, Fig. 1) is isolated as a naturally occurring aryltetralin lignan from *Podophyllum hexandrum*. Compound **1** is used as a lead compound for development of potent medicinal drugs and agrochemical agents.[1–4] Previously, we found that some esters of 2′(2′,6′)-(di)halogenopodophyllotoxins (**2**–**4**, Fig. 1) exhibited more pronounced insecticidal activity than toosendanin (Fig. 1, a botanical insecticide registered in China) isolated from *Melia azedarach*.[5] Once *C*2′-halogen atom is introduced into compound **1**, free rotation of its E-ring around C1-C1′ bond will be restricted (Fig. 1).[6] interestingly, it can result in no significant cytotoxicity of corresponding *C*2′-halogenopodophyllotoxins derivatives.[7] Cytisine (Fig. 1) is isolated from *Thermopsis lanceolata*, and some derivatives of 5 (3,5)-(di)halogenocytisines showed potent pesticidal activities.[8,9] On the other hand, products containing the succinic acid fragment usually displayed promising activities such as inhibitors of interleukin-1β converting enzyme and anti-tumor activity;[10,11] moreover, their characteristics such as the high-affinity binding and the deliverable ability were improved.[12,13]

Additionally, because of their high diversity and inherent biological activities, chemical structural optimization of natural products has been a fascinating approach to obtain pesticide candidates in recent years.[14–17] In the present paper, in continuation of our program to discover new potential pesticidal agents, therefore, twin compounds of podophyllotoxin and cytisine (**I**–**IV**, Fig. 1) were designed by insertion of the succinic acid fragment between 2′(2′,6′)-(di)halogenopodophyllotoxins and 5(3,5)-(di)halogenocytisines. Their insecticidal activity was tested against one threatening lepidopteran pest, *Mythimna separata* Walker.[18]

As depicted in Scheme 1, 2′(2′,6′)-(di)halogenopodophyllotoxins (**2**–**4**) were prepared in 81%–85% yields according to our previous method.[5] 5(3,5)-(Di)halogenocytisines (**9b**–**d**) were halogenated from cytisine (**9a**).[8] Then compounds **1**–**4** reacted with succinic anhydride in the presence of Et_3N and DMAP to give intermediates **5**–**8**.[19] Finally, compounds **5**–**8** reacting with different **9a**–**d** afforded podophyllotoxin-cytisine hydrids (**I**–**IV**) in 14%–60% yields.[20] Their structures were well characterized by melting points, optical rotation, IR, HRMS, and ^1H NMR (see Supplementary data).[21]

The insecticidal activity of compounds **1**–**9** and **I**–**IV** at 1 mg/mL was tested against the pre-third-instar larvae of *M. separata* by leaf-dipping method.[22,23] The toxic symptoms of *M. separata* treated by compounds **1**–**9** and **I**–**IV** were the same as our previous reports.[6,22,23] Among all derivatives, compounds **3**, **9c**, **9d**, **Ib, Ic, IIa**, **IIIb**, **IIIc**, **IIId**, **IVa**, **IVb** and **IVd** exhibited more potent insecticidal activity than toosendanin. The final mortality rates (FMRs) of compounds **3**, **9c**, **9d**, **Ib**, **Ic**, **IIa**, **IIIb**, **IIIc**, **IIId**, **IVa**, **IVb** and **IVd** against *M. separata* were 55.2%, 48.3%, 51.7%, 55.2%, 51.7%, 65.5%, 48.3%, 62.1%, 48.3%, 55.2%, 55.2%, 55.2%, and 62.1%, respectively; however, the FMRs of two lead compounds **1** and **9a**, and toosendanin were 31.0%, 37.9%, and 44.8%, respectively. Especially compounds **IIa** (X = Cl, Y = R^1 = R^2 = H), **IIIc** (X = Y = R^1 = R^2 = Cl) and **IVd** (X = R^1 = R^2 = Br, Y = H) showed the most pronounced insecticidal activity. 2′,6′-Dichloropodophyllotoxins (**3** and **7**) displayed more potent activity than that of podophyllotoxins (**1** and **5**), 2′-chloropodophyllotoxins (**2** and **6**) and 2′-bromopodophyllotoxins (**4** and **8**). It suggested that introduction of two chlorine atoms at the C-2′ and C-6′ positions of podophyllotoxin was necessary for the insecticidal activity

* Corresponding authors.
E-mail addresses: lvmin@nwsuaf.edu.cn (M. Lv), orgxuhui@nwsuaf.edu.cn (H. Xu).

https://doi.org/10.1016/j.bmcl.2021.128104
Received 11 March 2021; Received in revised form 26 April 2021; Accepted 5 May 2021
Available online 10 May 2021

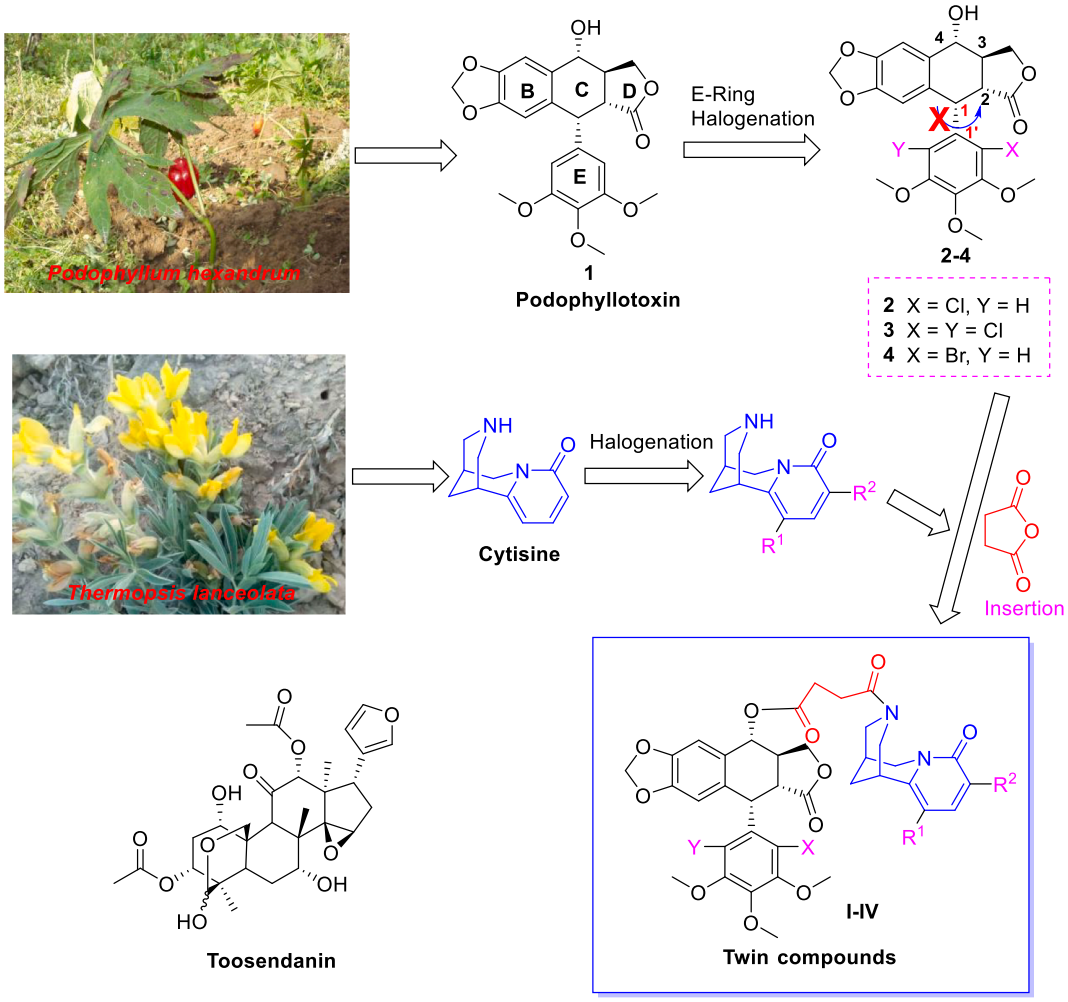
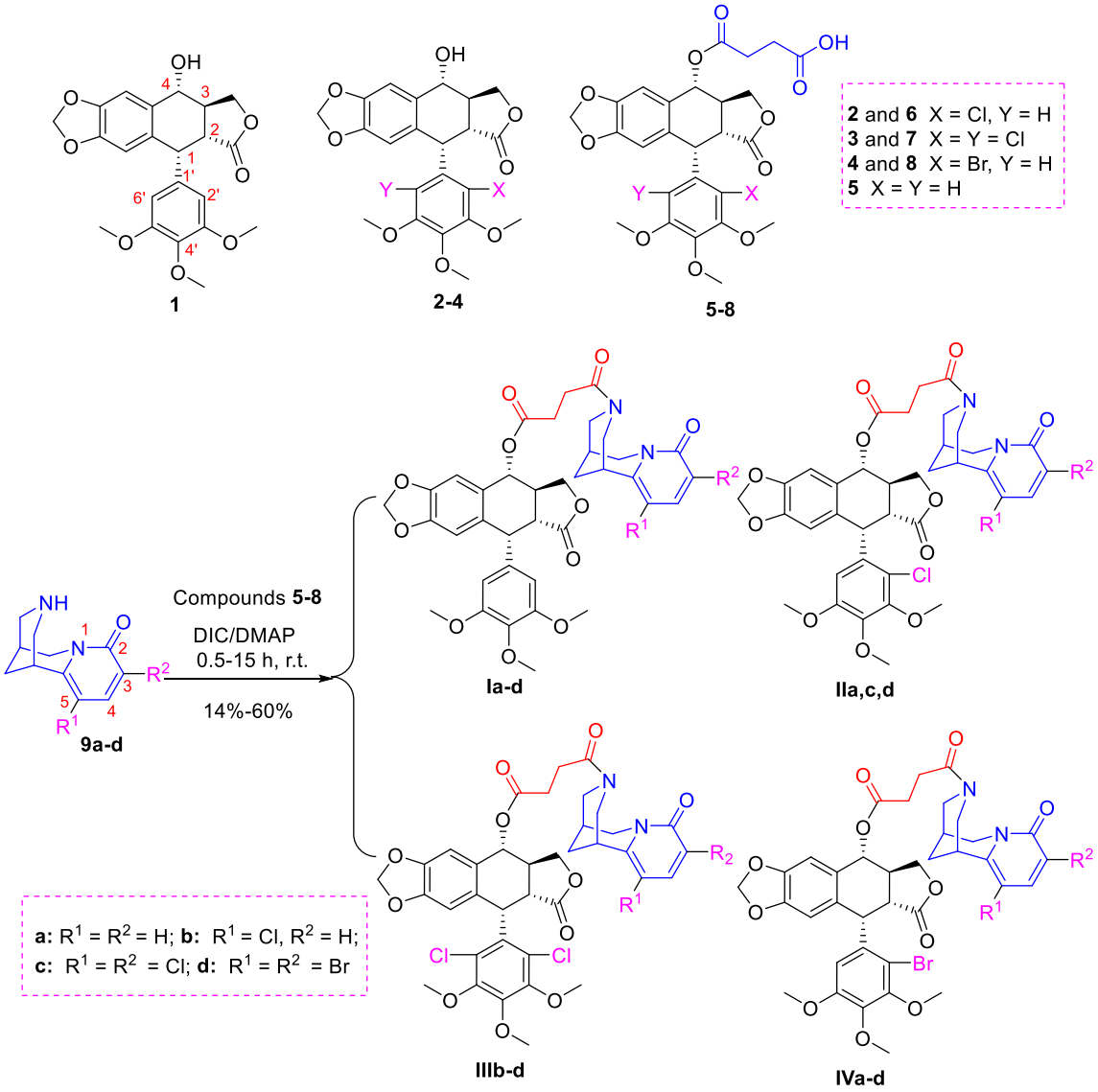


Fig. 1. Design of twin compounds from podophyllotoxin and cytisine isolated from *Podophyllum hexandrum* and *Thermopsis lanceolata*.



Scheme 1. Preparation of podophyllotoxin-cytisine twin insecticides (**I**–**IV**).

Table 1
Insecticidal activity of compounds **1**–**9** and **I**–**IV** against the pre-third-instar larvae of *M. separata*.

Compound	Final mortality rate (mean ± SE, %)
1	31.0 ± 3.3
2	44.8 ± 3.3
3	55.2 ± 3.3
4	37.9 ± 5.8
5	31.0 ± 3.3
6	34.5 ± 3.3
7	41.4 ± 3.3
8	31.0 ± 3.3
9a	37.9 ± 5.8
9b	41.4 ± 3.3
9c	48.3 ± 0
9d	51.7 ± 3.3
Ia	37.9 ± 0
Ib	55.2 ± 3.3
Ic	51.7 ± 3.3
Id	41.4 ± 3.3
IIa	65.5 ± 3.3
IIc	41.4 ± 6.7
IId	41.4 ± 3.3
IIIb	48.3 ± 0
IIIc	62.1 ± 3.3
IIId	48.3 ± 5.8
IVa	55.2 ± 3.3
IVb	55.2 ± 3.3
IVc	41.4 ± 3.3
IVd	62.1 ± 3.3
toosendanin	44.8 ± 3.3

(Table 1).

To podophyllotoxin-cytisine twins (**I**–**IV**), in general, the insecticidal activity of derivatives of 2′,6′-dichloropodophyllotoxin (**III**) and 2′-bromopodophyllotoxin (**IV**) was more potent than that of derivatives of podophyllotoxin (**I**) and 2′-chloropodophyllotoxin (**II**). To podophyllotoxin derivatives (**I**), introduction of the chlorine atom on the cytisine was important for the insecticidal activity (e.g., **Ib** and **Ic** Vs. **Ia** and **Id**); to 2′-chloropodophyllotoxin derivatives (**II**), however, introduction of the halogen atom on the cytisine was adverse to the insecticidal activity (e.g., **IIa** Vs. **IIc** and **IId**); to 2′,6′-dichloropodophyllotoxin derivatives (**III**), introduction of two chlorine atoms on the cytisine was necessary to the insecticidal activity (e.g., **IIIc** Vs. **IIIb** and **IIId**); To 2′-bromopodophyllotoxin derivatives (**IV**), however, introduction of two chlorine atoms on the cytisine was adverse to the insecticidal activity (e.g., **IVc** Vs. **IVa**, **IVb** and **IVd**). Therefore, the insecticidal activity of podophyllotoxin-cytisine hydrids (**I**–**IV**) was not only depended on the halogenation of podophyllotoxin, but also related to the halogenation of cytisine.

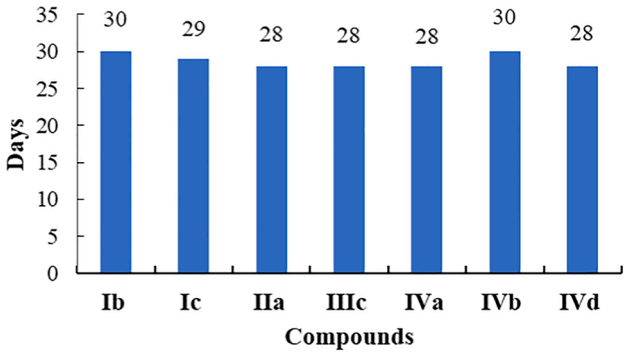


Fig. 3. Lethal time for 50% mortality of *M. separata* treated with compounds **Ib**, **Ic**, **IIa**, **IIIc**, **IVa**, **IVb** and **IVd**.

The percentages of FMRs at three growth stages of *M. separata* treated with compounds **Ib**, **Ic**, **IIa**, **IIIc**, **IVa**, **IVb**, **IVd** and toosendanin were shown in Fig. 2. Except compound **IVd**, the percentages of FMRs at the pupal stage of of *M. separata* treated with compounds **Ib**, **Ic**, **IIa**, **IIIc**, **IVa** and **IVb** were greater than 36.8%, and their largest parts of FMRs were at the pupal stage. But the largest part of FMR of of *M. separata* treated with toosendanin was at the larval stage. It suggested that compounds **Ib**, **Ic**, **IIa**, **IIIc**, **IVa** and **IVb** may show the different mechanism of action against *M. separata* when compared with toosendanin. As described in Fig. 3, the lethal time for 50% mortality of *M. separata* treated with compounds **Ib**, **Ic**, **IIa**, **IIIc**, **IVa**, **IVb** and **IVd** a was 30, 29, 28, 28, 28, 30, and 28 days, respectively. These were further demonstrated that these podophyllotoxin-cytisine hydrids showed delayed insecticidal activity against *M. separata*.

In conclusion, to discover potential pesticide candidates from bioactive natural products, twin insecticides **I**–**IV** were semi-prepared from cytisine and podophyllotoxin. Compounds **IIa**, **IIIc** and **IVd** displayed greater than 2-fold pronounced insecticidal activity of podophyllotoxin against armyworm. It demonstrated that introduction of two chlorine atoms at the C-2′ and C-6′ positions of podophyllotoxin was

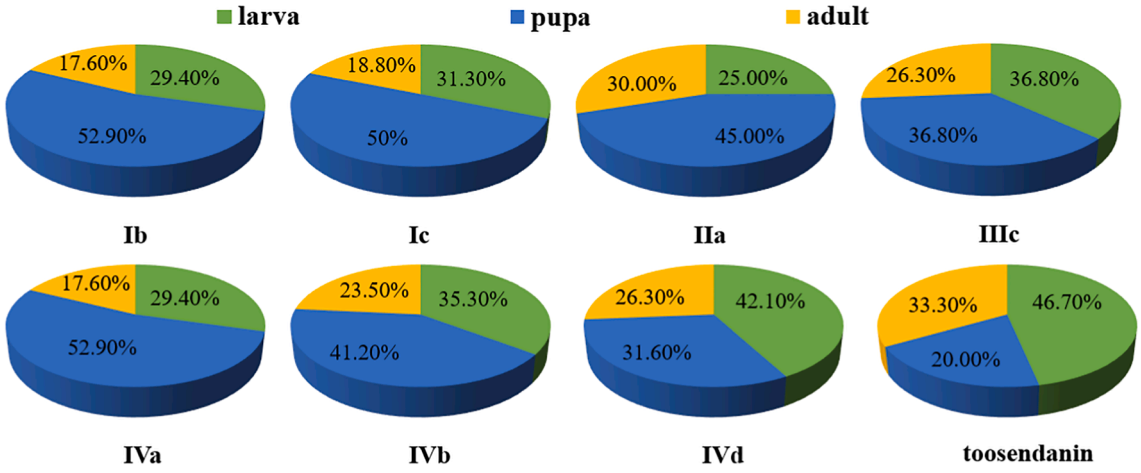


Fig. 2. The percentages of FMRa at three different growth stages of *M. separata* treated with compounds **Ib**, **Ic**, **IIa**, **IIIc**, **IVa**, **IVb**, **IVd** and toosendanin.

necessary for the insecticidal activity; the halogen atom played an important role for cytisines exhibiting good insecticidal activity; the insecticidal activity of these twin insecticides was related to the halogenation (including the halogen atoms and their number) of podophyllotoxin and cytisine. Most notably, the idea of twin insecticides for future high value-added application of natural plants in agriculture was addressed for the first time.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The present research was partly supported by National Natural Science Foundation of China (No. 31872013).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2021.128104.

References

1 Yu X, Che Z, Xu H. *Chem Eur J*. 2017;23:4467–4526.
2 Beauperin M, Polat D, Roudesly F, et al. *J Organometal Chem*. 2017;839:83–90.
3 Esfandiari M, Sharifi M, Mohamadyar-Toupkanlou F, et al. *Plant Cell Tiss Org*. 2018;133:51–61.
4 Nandagopal K, Halder M, Dash B, Nayak S, Jha S. *Curr Med Chem*. 2018;25:4693–4717.
5 Che ZP, Yu X, Zhi XY, Fan LL, Xu H. *J Agric Food Chem*. 2013;61:8148–8155.
6 Che ZP, Yu X, Fan LL, Xu H. *Bioorg Med Chem Lett*. 2013;23:5592–5598.
7 Hu H, Liu SY, Cheng YC, Lee KH, Wang ZQ. *J Med Chem*. 1989;35:866–871.
8 Huang X, Lv M, Xu H. *Pest Manag Sci*. 2019;75:2598–2609.
9 Huang X, Xu H. *Mini Rev Med Chem*. 2020;20:369–395.
10 Galatsis P, Caprathe B, Gilmore J, et al. *Bioorg Med Chem Lett*. 2010;20:5184–5190.
11 Zhou W, Cao J, Wang X, Guo J, Zhao Y. *Bioorg Med Chem Lett*. 2017;27:1076–1080.
12 Lam P, Lee KK, Wong RS, et al. *Bioorg Med Chem Lett*. 2012;22:3213–3218.
13 Molnár T, Visy J, Simon A, et al. *Bioorg Med Chem Lett*. 2008;18:6290–6292.
14 Hou QQ, Jing YF, Shao XS. *Chin Chem Lett*. 2017;28:1723–1726.
15 Zhu XL, Zhang R, Wu QY, et al. *J Agric Food Chem*. 2019;67:2774–2781.
16 Marrone PG. *Pest Manag Sci*. 2019;75:2325–2340.
17 Lv M, Sun ZQ, Li SC, Zhang S, Xu H. *Bioorg Med Chem Lett*. 2020;30, 127346.
18 Zhang B, Liu J, Chen X, Yuan G. *Entomol Res*. 2018;48:390–399.
19 Sk UH, Dixit D, Sen E. *Eur J Med Chem*. 2013;68:47–57.
20 Santos LS, Theoduloz C, Pilli RA, Rodriguez J. *Eur J Med Chem*. 2009;44:3810–3815.
21 Synthesis of podophyllotoxin-cytisine twin compounds (I–IV): A solution of compounds 5–8 (0.17 mmol), 9a–d (0.19 mmol), N,N'-diisopropylcarbodiimide (DIC, 0.24 mmol) and 4-dimethylaminopyridine (DMAP, 0.03 mmol) in dichloromethane (4 mL) was stirred at room temperature for 0.5–15 h. Then the mixture was diluted by ethyl acetate (30 mL), washed by 0.1 M aq. HCl (10 mL), 5% aq. NaHCO3 (10 mL) and brine (10 mL), dried over anhydrous Na2SO4, concentrated, and purified by preparative thin-layer chromatography (PTLC) to produce compounds I–IV in 14%–60% yields. Data for Ia: (two rotamers, ratio: 56:44): Yield: 51%, white solid, m.p. 154-156 oC; [α]20D = −42 (c 2.2 mg/mL, CHCl3); IR cm-1 (KBr): 2931, 1735, 1651, 1579, 1479, 1233, 1124, 996, 867, 799; 1H NMR (500 MHz, CDCl3) δ: 7.27–7.30 (m, 1H, H-4''), 6.80 (s, 0.56H, H-5), 6.77 (s, 0.44H, H-5), 6.50 (s, 1H, H-8), 6.44–6.48 (m, 1H, H-3''), 6.37 (s, 2H, H-2', 6'), 6.05–6.08 (m, 1H, H-5''), 5.95–5.97 (m, 2H, OCH2O), 5.85 (d, J = 6.0 Hz, 0.56H, H-4), 5.83 (d, J = 6.0 Hz, 0.44H, H-4), 4.80 (d, J = 13.0 Hz, 0.44H), 4.66 (d, J = 13.0 Hz, 0.56H), 4.57 (d, J = 4.0 Hz, 1H, H-1), 4.33–4.36 (m, 0.45H), 4.14–4.22 (m, 1.58H), 3.99–4.09 (m, 2H), 3.88–3.93 (m, 1H), 3.81 (s, 3H, OCH3), 3.76 (s, 3H, OCH3), 3.74 (s, 3H, OCH3), 3.44 (d, J = 12.5 Hz, 0.44H), 3.39 (d, J = 12.5 Hz, 0.56H), 3.10 (s, 0.44H, H-7''), 3.08 (s, 0.56H, H-7''), 2.77–2.92 (m, 3H), 2.65–2.72 (m, 1H), 2.45–2.62 (m, 4H), 2.03–2.10 (m, 2H, H-8''). HRMS (ESI): Calcd for C37H38N2O11Na ([M+Na]+), 709.2367; found, 709.2345.
22 Yang RG, Zhang YY, Xu H. *Bioorg Med Chem Lett*. 2018;28:1410–1416.
23 Hao M, Sun ZQ, Xu JW, Lv M, Xu H. *J Agric Food Chem*. 2020;68:4131–4143.