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

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ABSTRACT

By using podophyllotoxin as a lead compound, eight novel esters of 2-chloropodophyllotoxin were designed, semisynthesized, and preliminarily evaluated for their insecticidal activity against the pre-third-instar larvae of *Mythimna separata* Walker in vivo for the first time. Among all the tested compounds, especially three esters of 2-chloropodophyllotoxin **8a**, **8c**, and **8g**, and one intermediate **6** showed more promising and pronounced insecticidal activity than toosendanin, a commercial insecticide derived from *Melia azedarach*.

Podophyllotoxin (**1**, Fig. 1), one of the well-known naturally occurring aryltetralin lignans, is extracted as the main component from the roots and rhizomes of *Podophyllum* species such as *Podophyllum hexandrum* and *Podophyllum peltatum*.[1] Its derivatives, such as etoposide (VP-16, **2**), teniposide (VM-26, **3**), and etopophos (etoposide phosphate, **4**), have been used as DNA topoisomerase II inhibitors in chemotherapy for various types of cancer,[2] and they are also effective in the treatment of human immunodeficiency virus (HIV).[3] Recently, we have reported the insecticidal activity of the podophyllotoxin derivatives and found some compounds showed more promising insecticidal activity than podophyllotoxin.[4,5] According to the previous structure–insecticidal activity relationship, it was found that the *trans*-lactone and 4′-OCH_3 moieties of podophyllotoxin were essential to maintain the insecticidal activity.[6] Meanwhile, Durst and co-workers described that 2-chloropodophyllotoxin had significant anticancer activity.[7] Based upon the above results, we want to further study the insecticidal activity of 2-chloropodophyllotoxin derivatives being the *trans*-lactone and 4′-OCH_3 fragments. Therefore, as an important part of our program

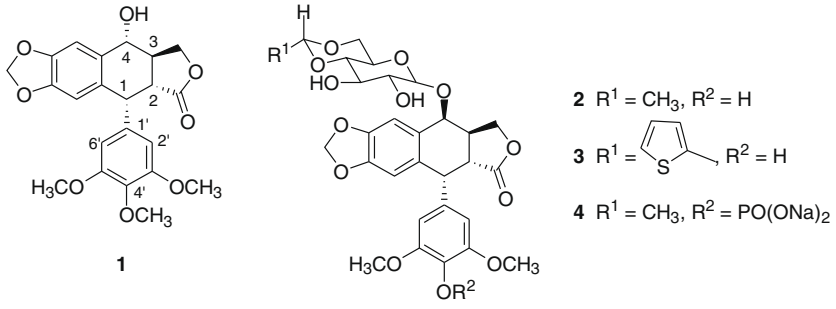

Figure 1.

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aimed at the discovery and development of bioactive molecules,[4,5,8–12] in this Letter a series of novel esters of 2-chloropodophyllotoxin (**8a**–**8h**) were designed, semisynthesized, and preliminarily evaluated for their insecticidal activity against the pre-third-instar larvae of *Mythimna separata* Walker in vivo for the first time.

A series of novel esters of 2-chloropodophyllotoxin (**8a**–**8h**) were synthesized as shown in Scheme 1. Starting from podophyllotoxin (**1**), the 4-OH group of **1** was firstly protected by a tetrahydropyranyl (THP) group in the presence of phosphorus oxychloride and dihydropyran (DHP) to give 4-*O*-tetrahydropyranylpodophyllotoxin (**5**).[13] Then 2-chloro-4-*O*-tetrahydropyranylpodophyllotoxin (**6**) was obtained by treatment of **5** with lithium diisopropylamide (LDA) at −78 °C in dry THF, and followed by stereoselective reaction with hexachloroethane. Subsequently, hydrolysis of the THP group of **6** to afford 2-chloropodophyllotoxin (**7**).[7] Finally, eight novel esters of 2-chloropodophyllotoxin (**8a**–**8h**) were obtained by reaction of **7** with the corresponding aromatic acids in the presence of diisopropylcarbodiimide (DIC) and 4-dimethylaminopyridine (DMAP). The structures of the compounds were well characterized by ^{1}H NMR, HRMS, MS, optical rotation, and IR.[14]

In order to obtain precise three-dimensional structural information and absolute configuration of **8a**–**8h**, compound **8h** was recrystallized by slow evaporation from methanol and its single-crystal structure was determined by X-ray crystallography as illustrated in Figure 2.[15] It was clearly demonstrated that the 2-chloro and the 4-ester groups of **8h** adopted the β- and α-configuration, respectively.

The insecticidal activity of compounds **5**–**7** and **8a**–**8h** against the pre-third-instar larvae of *M. separata* in vivo was screened by the leaf-dipping method at the concentration of 1 mg/mL.[4] Podophyllotoxin phenylacetate (**9**),[16] and toosendanin, a commercial insecticide derived from *Melia azedarach*, were used as positive controls.

As indicated in Table 1, the corresponding corrected mortality rates caused by these compounds after 35 d were far higher than those after 10 and 20 d. For example, the corrected mortality rate of **8a** against *M. separata* after 10 d was only 10.7%, after 20 d the corresponding mortality rate was increased to 32.1%, but after 35 d the corresponding mortality rate was rapidly increased to 70.4%, which was nearly seven times of the mortality rate after

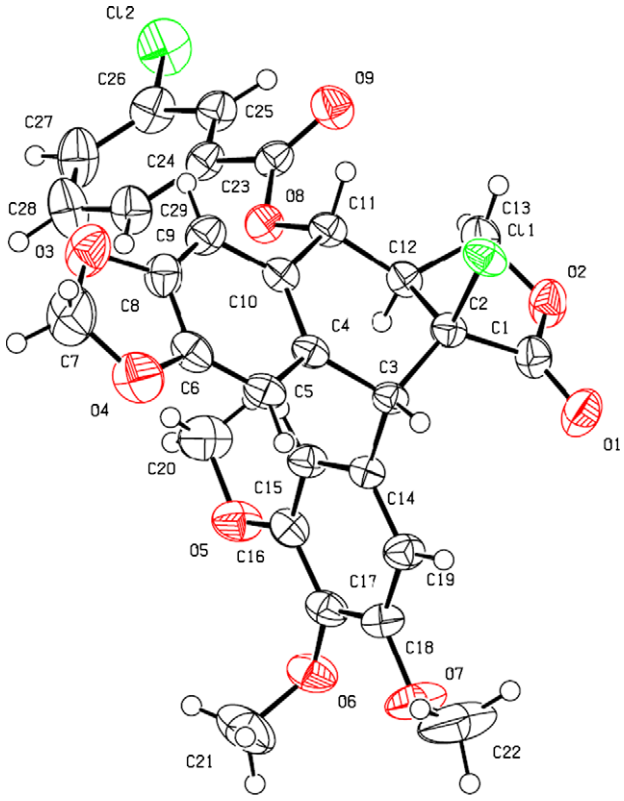
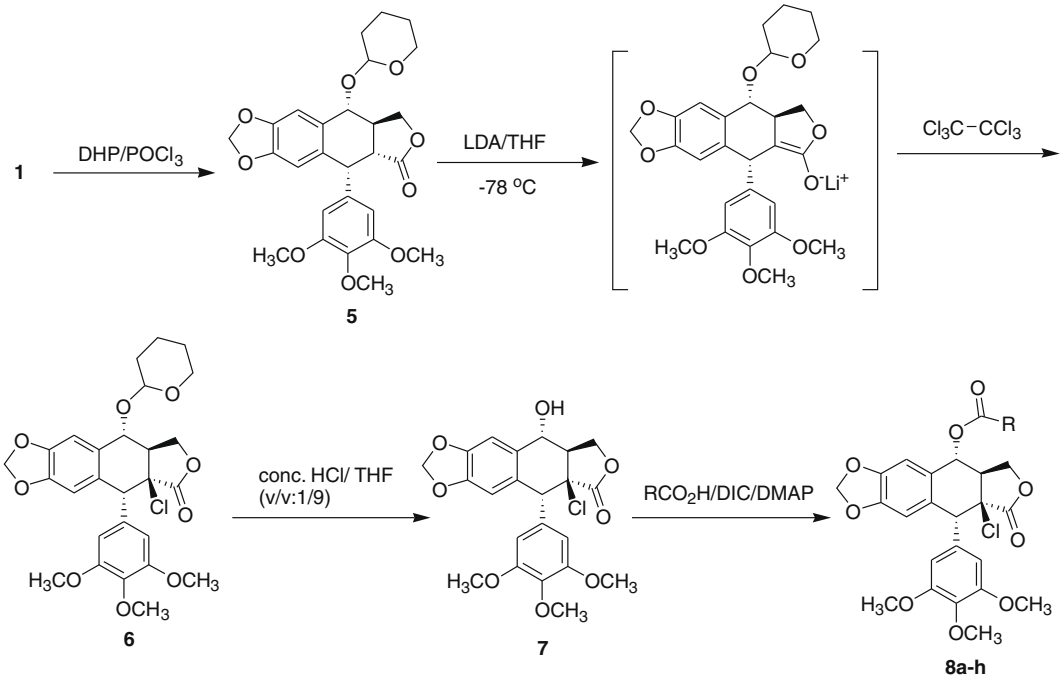
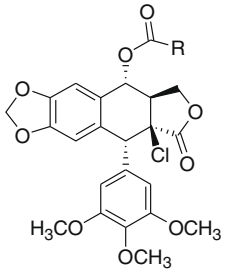


Figure 2. The X-ray crystallography of compound **8h**.

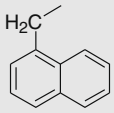
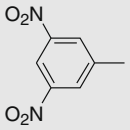
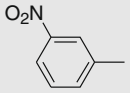
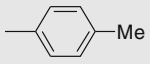
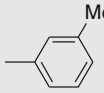
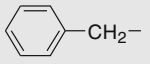
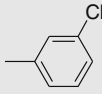


Scheme 1.

Table 1
Insecticidal activity of novel esters of 2-chloropodophyllotoxin (**8a**–**8h**) against *Mythimna separata* Walker in vivo



8a-h

Compounds	R	Corrected mortality rate (%)		
		10 d	20 d	35 d
1	/	17.9 (±17.0)	17.9 (±17.0)	44.4 (±12.5)
5	/	10.7 (±9.4)	14.3 (±14.1)	51.9 (±12.5)
6	/	23.3 (±12.5)	33.3 (±4.7)	66.7 (±4.7)
7	/	7.14 (±4.7)	17.9 (±12.5)	37.0 (±9.4)
8a		10.7 (±12.5)	32.1 (±4.7)	70.4 (±4.7)
8b		10.7 (±9.4)	25.0 (±14.1)	44.4 (±16.3)
8c		28.6 (±12.5)	50.0 (±4.7)	63.0 (±4.7)
8d		10.7 (±12.5)	21.4 (±12.5)	40.7 (±9.4)
8e		3.6 (±8.2)	3.6 (±8.2)	40.7 (±17.0)
8f		10.7 (±4.7)	21.4 (±9.4)	44.4 (±14.1)
8g		28.6 (±9.4)	32.14 (±12.5)	63.0 (±12.5)
8h		7.1 (±4.7)	14.3 (±8.2)	40.7 (±12.5)
9	/	3.6 (±0)	17.9 (±12.5)	51.9 (±9.4)
Toosendanin	/	25.0 (±0)	35.7 (±8.2)	51.9 (±12.5)

10 d. That is, these compounds, different from those conventional neurotoxic insecticides, such as organophosphates, carbamates, and pyrethroids, showed delayed insecticidal activity.[4,5] Meanwhile, the symptoms of the tested *M. separata* were also characterized as the same as our previous reports.[4,5] 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 to 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.

Some preliminary structure–insecticidal activity relationships on the 2-chloropodophyllotoxin derivatives were also investigated. As shown in Table 1, when α-naphthylacetyl, 3-nitrobenzoyl, or phenylacetyl group was introduced on the C-4 position of 2-chloropodophyllotoxin, the corresponding esters **8a**, **8c**, and **8g** showed more potent insecticidal activity than toosendanin. For example, the final mortality rates of **8a**, **8c**, and **8g** were 70.4%, 63.0%, and 63.0%, respectively, while the final mortality rate of toosendanin was 51.9%. However, when 3,5-dinitrobenzoyl, 4-methylbenzoyl, 3-methylbenzoyl, 2-chlorobenzoyl, or 3-chlorobenzoyl group was introduced on the C-4 position of 2-chloropodophyllotoxin, the final mortality rates of the corresponding esters **8b**, **8d**, **8e**, **8f**, and **8h** were ⩽44.4%, which were less potent than toosendanin. Interestingly, 2-chloro-4-*O*-tetrahydropyranylpodophyllotoxin (**6**), an intermediate of **8a**–**8h**, also exhibited more potent insecticidal activity than toosendanin (66.7% vs 51.9%), and this encouraging result will prompt us to further study the ether derivatives of 2-chloropodophyllotoxin as the insecticidal agent in future. Meanwhile, it was also found that the substituents effect on the C-4 position of 2-chloropodophyllotoxin derivatives was very important for their insecticidal activity: (1) When some substituents were introduced on the C-4 position of 2-chloropodophyllotoxin, the corresponding compounds usually showed more potent insecticidal activity than the podophyllotoxin ones. For example, 2-chloro-4-*O*-tetrahydropyranylpodophyllotoxin (**6**) exhibited more potent insecticidal activity than 4-*O*-tetrahydropyranylpodophyllotoxin (**5**) (66.7% vs 51.9%), and 2-chloropodophyllotoxin phenylacetate (**8g**) showed more potent insecticidal activity than the corresponding podophyllotoxin phenylacetate (**9**) (63.0% vs 51.9%). (2) When there was no substituent introduced on the C-4 position of 2-chloropodophyllotoxin, the corresponding compound showed less potent insecticidal activity than podophyllotoxin (37.0% for **7** vs 44.4% for **1**). Consequently, in combination of our previous results that introducing 4β-benzenesulfonamide substituents on the C-4 position of podophyllotoxin could lead to more potent compounds,[5] some 4β-benzenesulfonamide-2-chloropodophyllotoxin derivatives will be synthesized and studied in our laboratory.

In conclusion, eight novel esters of 2-chloropodophyllotoxin (**8a**–**8h**) were designed, semisynthesized, and evaluated for their insecticidal activity against the pre-third-instar larvae of *M. separata* in vivo at the concentration of 1 mg/mL. Among all the tested compounds, especially three esters of 2-chloropodophyllotoxin **8a**, **8c**, and **8g**, and one intermediate **6** exhibited the more promising and pronounced insecticidal activity than toosendanin, a commercial insecticide derived from *M. azedarach*.

Acknowledgments

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14. *Spectral data for* **8a**: White solid, mp 200–203 °C; $[\alpha]_D^{20} = -147$ (*c* 0.56 mg/mL, $CHCl_3$); IR cm^{-1}: 2919, 1776, 1736, 1596, 1505, 1484, 1459, 1379, 1236, 1127, 1038, 937, 753; ^1H NMR (400 MHz, $CDCl_3$) δ: 7.98 (d, *J* = 8.0 Hz, 1H, naphthalene ring), 7.88 (d, *J* = 7.2 Hz, 1H, naphthalene ring), 7.82 (dd, *J* = 2.8, 6.8 Hz, 1H, naphthalene ring), 7.53 (m, 2H, naphthalene ring), 7.41 (m, 2H, naphthalene ring), 6.54 (s, 1H, H-5), 6.48 (s, 1H, H-8), 6.32 (s, 2H, H-2′, 6′), 5.97 (m, 3H, H-4 and OCH_2O), 4.71 (s, 1H, H-1), 4.35 (m, 1H, H-11), 4.19 (s, 2H, $CH_2C_{10}H_7$), 4.05 (m, 1H, H-11), 3.78 (s, 3H, 4′-OCH_3), 3.63 (s, 6H, 3′,5′-OCH_3), 2.93 (m, 1H, H-3); ESI-MS *m/z*: 639 ($[M+Na]^+$, 78), 641 ($[M+Na]^+$, 33); HRMS: Anal. Calcd for $C_{35}H_{31}O_9NaCl$ ($[M+Na]^+$): 639.1392. Found: 639.1396. Compound **8b**: Yellow solid, mp 142–145 °C; $[\alpha]_D^{20} = -90$ (*c* 0.26 mg/mL, $CHCl_3$); IR cm^{-1}: 2923, 2845, 1787, 1732, 1590, 1493, 1464, 1337, 1245, 1119, 1041, 927, 781, 721; ^1H NMR (400 MHz, $CDCl_3$) δ: 9.30 (s, 1H, H-4″), 9.15 (s, 2H, H-2″, 6″), 6.82 (s, 1H, H-5), 6.64 (s, 1H, H-8), 6.50 (s, 2H, H-2′, 6′), 6.35 (d, *J* = 8.8 Hz, 1H, H-4), 6.04 (m, 2H, OCH_2O), 4.86 (s, 1H, H-1), 4.62 (m, 1H, H-11), 4.41 (m, 1H, H-11), 3.75 (s, 9H, 3′,4′,5′-OCH_3), 3.31 (m, 1H, H-3); ESI-MS *m/z*: 665 ($[M+Na]^+$, 20), 667 ($[M+Na]^+$, 9); HRMS: Anal. Calcd for $C_{29}H_{23}N_2O_{13}NaCl$ ($[M+Na]^+$): 665.0781. Found: 665.0776. Compound **8c**: Yellow solid, mp 96–98 °C; $[\alpha]_D^{20} = -103$ (*c* 0.38 mg/mL, $CHCl_3$); IR cm^{-1}: 2936, 2831, 1786, 1726, 1588, 1532, 1504, 1484, 1459, 1380, 1237, 1123, 1036, 932, 772, 718; ^1H NMR (400 MHz, $CDCl_3$) δ: 8.82 (s, 1H, H-2″), 8.40 (d, *J* = 7.6 Hz, 2H, H-4″, 6″), 7.72 (m, *J* = 8.0 Hz,1H, H-5″), 6.86 (s, 1H, H-5), 6.62 (s, 1H, H-8), 6.50 (s, 2H, H-2′, 6′), 6.28 (d, *J* = 9.2 Hz, 1H, H-4), 6.03 (m, 2H, OCH_2O), 4.85 (s, 1H, H-1), 4.60 (m, 1H, H-11), 4.42 (m, 1H, H-11), 3.80(s, 9H, 3′,4′,5′-OCH_3), 3.24 (m, 1H, H-3); ESI-MS *m/z*: 620 ($[M+Na]^+$, 100), 622 ($[M+Na]^+$, 42); HRMS: Anal. Calcd for $C_{29}H_{28}ClN_2O_{11}$ ($[M+NH_4]^+$): 615.1376. Found: 615.1370. Compound **8d**: White solid, mp 92–94 °C; $[\alpha]_D^{20} = -108$ (*c* 0.36 mg/mL, $CHCl_3$); IR cm^{-1}: 2917, 2831, 1784, 1720, 1588, 1504, 1485, 1455, 1380, 1239, 1122, 1101, 1034, 929,752; ^1H NMR (400 MHz, $CDCl_3$) δ: 7.91 (d, *J* = 8.0 Hz,2H, H-2″, 6″), 7.26 (d, *J* = 2.8 Hz,5H, H-3″, 5″ and 4″-CH_3), 6.91 (s, 1H, H-5), 6.59 (s, 1H, H-8), 6.49 (s, 2H, H-2′, 6′), 6.18 (d, *J* = 9.2 Hz, 1H, H-4), 6.00 (m, 2H, OCH_2O), 4.83 (s, 1H, H-1), 4.63 (m, 1H, H-11), 4.44 (m, 1H, H-11), 3.71(s, 9H, 3′,4′,5′-OCH_3), 3.23 (m, 1H, H-3); ESI-MS *m/z*: 589 ($[M+Na]^+$, 8), 591 ($[M+Na]^+$, 3); HRMS: Anal. Calcd for $C_{30}H_{27}KClO_9$ ($[M+K]^+$): 605.0975. Found: 605.0987. Compound **8e**: White solid, mp 116—119 °C; $[\alpha]_D^{20} = -128$ (*c* 0.33 mg/mL, $CHCl_3$); IR cm^{-1}: 2931, 2821, 1786, 1735, 1588, 1503, 1484, 1458, 1378, 1237, 1124, 1037, 934, 775, 721; ^1H NMR (400 MHz, $CDCl_3$) δ: 7.83 (s, 2H, H-2″, 6″), 7.43 (d, *J* = 7.2 Hz, 1H, H-5″), 7.36 (m, *J* = 8.0 Hz, 1H, H-4″), 6.91 (s, 1H, H-5), 6.60 (s, 1H, H-8), 6.50 (s, 2H, H-2′, 6′), 6.21 (d, *J* = 8.8 Hz, 1H, H-4), 6.00 (m, 2H, OCH_2O), 4.83 (s, 1H, H-1), 4.61 (m, 1H, H-11), 4.43 (m, 1H, H-11), 3.80 (s, 9H, 3′,4′,5′-OCH_3), 3.26 (m, 1H, H-3), 2.41 (s, 3H, 3″-CH_3); ESI-MS *m/z*: 589 ($[M+Na]^+$, 100), 591 ($[M+Na]^+$, 38); HRMS: Anal. Calcd for $C_{30}H_{27}NaClO_9$ ($[M+Na]^+$): 589.1236. Found: 589.1229. Compound **8f**: White solid, mp 202—205 °C; $[\alpha]_D^{20} = -121$ (*c* 0.30 mg/mL, $CHCl_3$); IR cm^{-1}: 2929, 2831, 1781, 1731, 1590, 1502, 1482, 1454, 1380, 1235, 1122, 1032, 925, 749; ^1H NMR (400 MHz, $CDCl_3$) δ: 7.85 (d, *J* = 8.0 Hz, 1H, H-6″), 7.49 (m, 2H, H-3″, 5″), 7.35 (m, 1H, H-4″), 6.94 (s, 1H, H-5), 6.58 (s, 1H, H-8), 6.47 (s, 2H, H-2′, 6′), 6.28 (d, *J* = 9.2 Hz, 1H, H-4), 6.00 (m, 2H, OCH_2O), 4.82 (s, 1H, H-1), 4.63 (m, 1H, H-11), 4.49 (m, 1H, H-11), 3.74(s, 3H, 4′-OCH_3), 3.68(s, 6H, 3′,5′-OCH_3), 3.30(m, 1H, H-3); ESI-MS *m/z*: 609 ($[M+Na]^+$, 100), 611 ($[M+Na]^+$, 75), 613 ($[M+Na]^+$, 16); HRMS: Anal. Calcd for $C_{29}H_{24}NaCl_2O_9$ ($[M+Na]^+$): 609.0690. Found: 609.0689. Compound **8g**: White solid, mp 88–91 °C; $[\alpha]_D^{20} = -150$ (*c* 0.54 mg/mL, $CHCl_3$); IR cm^{-1}: 2923, 2831, 1780,1735, 1589, 1503, 1483, 1457, 1378, 1235, 1124, 1037, 935, 756; ^1H NMR (400 MHz, $CDCl_3$) δ: 7.36–7.26 (m, 5H, H-2″, 3″, 4″, 5″, 6″), 6.68 (s, 1H, H-5), 6.52 (s, 1H, H-8), 6.39 (s, 2H, H-2′, 6′), 5.98 (m, 3H, H-4 and OCH_2O), 4.75 (s, 1H, H-1), 4.41 (m, 1H, H-11), 4.19 (m, 1H, H-11), 3.81–3.67(s, 11H, 3′, 4′, 5′-OCH_3 and $C_6H_5CH_2$), 3.03 (m, 1H, H-3); ESI-MS *m/z*: 589 ($[M+Na]^+$, 100), 591 ($[M+Na]^+$, 37); HRMS: Anal. Calcd for $C_{30}H_{27}NaClO_9$ ($[M+Na]^+$): 589.1236. Found: 589.1225. Compound **8h**: White solid, mp 82–84 °C; $[\alpha]_D^{20} = -99$ (*c* 0.71 mg/mL, $CHCl_3$); IR cm^{-1}: 2934, 2831, 1783, 1722, 1589, 1504, 1484, 1459, 1380, 1239, 1123, 1036, 933, 748; ^1H NMR (400 MHz, $CDCl_3$) δ: 7.98 (s, 1H, H-2″), 7.93 (d, *J* = 8.0 Hz, 1H, H-6″), 7.60 (d, *J* = 7.6 Hz, 1H, H-4″), 7.42 (m, 1H, H-5″), 6.88 (s, 1H, H-5), 6.60 (s, 1H, H-8), 6.49 (s, 2H, H-2′, 6′), 6.29 (d, *J* = 9.2 Hz, 1H, H-4), 6.01 (m, 2H, OCH_2O), 4.83 (s, 1H, H-1), 4.61 (m, 1H, H-11), 4.42 (m, 1H, H-11), 3.71(s, 9H, 3′,4′,5′-OCH_3), 3.25 (m, 1H, H-3); ESI-MS *m/z*: 609 ($[M+Na]^+$, 100), 611 ($[M+Na]^+$, 64), 613 ($[M+Na]^+$, 13); HRMS: Anal. Calcd for $C_{29}H_{24}O_9NaCl_2$ ($[M+Na]^+$): 609.0690. Found: 609.0685.
15. Crystallographic data (excluding structure factors) for the structure of **8h** in this paper has been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC 738791. 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].
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