Iridoid glycosides from *Callicarpa nudiflora* Hook

Shi-Xiu Feng\(^a\), Bo Yi\(^b\), Min Zhang\(^a\), Jing Xu\(^a\), Hai Lin\(^b\) and Wen-Tong Xu\(^b\)

\(^a\)Key Laboratory of South Subtropical Plant Diversity, Fairy Lake Botanical Garden, Shenzhen & Chinese Academy of Sciences, Shenzhen, China; \(^b\)Pharmacy Department of the 187th Hospital of People’s Liberation Army, Haikou, China

**ABSTRACT**

Four new iridoid glycoside derivatives (1–4), along with ten known iridoid glycosides (5–14), were isolated from *Callicarpa nudiflora* Hook et Arn. The structure of the new iridoid glycosides was elucidated as 5″-methoxy-ampicoside (1), 6″-O-trans-caffeoylcatalpol (2), 6″-O-trans-feruloylcatalpol (3) and 3″-methoxy-agnucastoside C (4) on the basis of spectroscopic analysis. Compounds 1–11 were reported from this plant for the first time. The cytotoxic activity of the isolated compounds against human cervical carcinoma Hela cells and ovarian carcinoma HeyA8 cells was evaluated using the microculture tetrazolium assay. Compounds 4, 5, 8, 12 and 13 showed cytotoxic activity against the Hela cell line with IC\(_{50}\) values of 25.3, 48.1, 17.3, 38.3 and 28.2 μM, respectively. While only compound 8 showed cytotoxicity against the HeyA8 cell line, with an IC\(_{50}\) of 35.5 μM.

1. Introduction

*Callicarpa nudiflora* Hook et Arn., belonging to the family of Verbenaceae, is widely distributed from southern China to Malaysia (Dong et al. 2014). This plant has been used as folk medicine in China for eliminating stasis to subdue swelling, treating respiratory tract infections, hepatitis, antiphlogosis, haemostasis and memory promote function. Phytochemical investigations of this plant led to the isolation of diterpenoids, triterpenoids, flavonoids,
phenylpropanoid glycosides and phenolic acids (Liang et al. 2011; Luo et al. 2014; Hjørnevik et al. 2015). However, cytotoxicity studies on iridoids have rarely been reported.

During our current work, a series of iridoid glycosides were obtained from this plant system, including four new iridoid glycoside derivatives 5′″-methoxy-ampicoside (1), 6″-O-trans-caffeoylcatalpol (2), 6′″-O-trans-feruloylcatalpol (3) and 3″-methoxy-agnucastoside C (4) (Figure 1), along with ten known iridoid glycosides 10-O-(E)-p-coumaroylgeniposidic acid (5) (Shaker et al. 2001), ampicoside (6) (Wang et al. 1993), 6-O-trans-feruloylcatalpol (7) (Li et al. 1993), agnucastoside C (8) (Kuruüzüm-Uz et al. 2003), ajugol (9) (Zhang 2013), 6-O-vanilloylajugol (10) (Akdemir et al. 2004), 6-O-syringoylajugol (11) (Warashina et al. 1991), linearoside (12) (Bergeron et al. 1997), nudifloside (13) (Mei et al. 2010) and catalpol (14) (Zhang 2013), of which compounds 1–11 were reported here as *C. nudiflora* compounds for the first time. Here, we report the isolation and structure elucidation of compounds 1–4 and the cytotoxic evaluation of compounds 1–14 against Hela and HeyA8 cancer cell lines.

2. Results and discussion

Compound 1 was isolated as a white amorphous powder, the molecular formula of C_{24}H_{30}O_{14} was established from a positive molecular ion peak at m/z 565.1520 [M + Na]^+ (Calcd for C_{24}H_{30}NaO_{14}: 565.1528) in the HR-ESI-MS spectrum and supported by the ESI–MS (m/z 565.2 [M + Na]^+, m/z 541.8 [M – H]^− and m/z 1083.3 [2M – H]^−) spectrum. Analysis of the ^1H and ^13C NMR spectroscopic data of 1 and comparison with spectra of known catalpol (Zhang 2013) showed that 1 could be such a compound. Furthermore, the chemical shift of the olefinic protons at δ_H 6.43 (1H, dd, J = 6.0 and 1.5 Hz, H-3) and δ_H 4.99 (1H, dd, J = 5.5 and

Figure 1. Chemical structure of new compounds 1–4.
5.0 Hz, H-4), the following H-atom of δ_H 5.12 (1H, d, J = 9.5 Hz, H-1), δ_H 2.61 (1H, m, H-5), δ_H 5.07 (1H, brd, J = 8.0 Hz, H-6), δ_H 3.71 (1H, brs, H-7), the glucopyranosyl signals δ_H 4.62 (1H, d, J = 8.0 Hz, glc-H-1’) and the C-atom signals at δ_C 58.7 (C-7) and δ_C 66.4 (C-8) were in good agreement with this. Thus, the above information deduced that the iridoid skeleton of 1 could be catalpol (Zhang 2013).

Moreover, the 1H NMR spectrum displayed signals for two aromatic protons at δ_H 7.25 (2H, s, H-2″,6″), indicated the presence of a 1,3,4,5-tetrasubstituted benzene ring. And the two methoxyl groups at δ_H 3.83 (6H, s, 3″ and 5″-OCH_3) corresponding to δ_C 56.6 was also observed on the bases of HSQC spectrum. In the HMBC spectrum (Figure 2), the correlations from OCH_3 to C-3″ and C-5″ suggested that the two methoxyl groups were linked to C-3″ and C-5″, respectively. Besides, a signal for carbonyl at δ_C 166.1 (C-7″) was presented in the 13C NMR. The further correlation signals from H-2″ and H-6″ to C-7″ observed in HMBC spectrum suggested the carbonyl is located at C-1″. Meanwhile, in the HMBC spectrum, the correlation from H-6 to C-7″ confirmed that carbonyl connects with the C-6 position of catalpol through ester linkage. Thus, the structure of 1 was similar to the iridoid glycoside ampicoside except for the presence of methoxyl group at C-5″ (Wang et al. 1993). Concluded all the information above, the structure of 1 was established as 5″-methoxy-ampicoside.

Compound 2 was obtained as a white amorphous powder and the positive ion HR-ESI-MS provided an ion m/z 547.1435 [M + Na]^+, corresponding to a molecular formula of C_{24}H_{28}O_{13} (Calcd for C_{24}H_{28}NaO_{13}: 547.1422), which was supported by m/z 547.3 [M + Na]^+, 1071.4 [2M + Na]^+, 523.9 [M − H]^− and 1047.5 [2M − H]^− in the ESI–MS spectrum. The 1H and 13C NMR spectroscopic data of 2 and comparison with spectra of known compounds showed that 2 was again an ester of catalpol. The chemical shifts for the 6′-CH_2 group at δ_H 4.51 (1H, dd,
J = 12.0 and 1.6 Hz, H-6’a) and 4.44 (1H, dd, J = 12.0 and 5.6 Hz, H-6’b) showed that this was the position of esterification. The remaining signals in the spectrum were typical for a caffeoyl group, and the connection to the catalpol moiety was confirmed by the correlation from H-6’b to C-9” in the HMBC spectrum. Therefore, the structure of 2 was deduced as 6”-O-trans-caffeoyl-catalpol.

Compound 3, a white amorphous powder, was assigned as C_{25}H_{30}O_{13} on the basis of its HR-ESI-MS data at m/z 561.1596 [M + Na]^+ (Calcd for C_{25}H_{30}NaO_{13}: 561.1579) and its ESI-MS data m/z 561.2 [M + Na]^+ and m/z 1075.3 [2M − H]^−. It was apparent from the NMR spectroscopic data (1H, 13C, COSY, HSQC, HMBC and NOESY) that this compound differed from 2 only for an additional methoxyl group. In the 13C NMR spectrum, the C-atom signal at δ\(_{C}\) 149.4 (C-3”) significantly shift to low field compared with 2 (δ\(_{C}\) 145.9) and the C-atom signal at δ\(_{C}\) 56.5 was presented, which indicated that the methoxyl group instead of the hydroxyl group in compound 3. In addition, the correlation signal between CH\(_{3}\)O-3” at δ\(_{H}\) 3.90 and C-3” was observed in HMBC spectrum, which suggested the methoxy group is located at C-3”. So, the structure of 3 was deduced as 6”-O-trans-feruloylcatalpol.

Compound 4 was obtained as a white amorphous powder, and the HR-ESI-MS spectrum showed a positive ion [M + Na]^+ at m/z 721.2089, in consistent with the molecular formula C\(_{35}\)H\(_{38}\)O\(_{15}\) (Calcd for C\(_{35}\)H\(_{38}\)NaO\(_{15}\): 721.2103), which was supported by the ESI-MS (m/z 699.2 [M + H]^+, m/z 721.2 [M + Na]^+, m/z 1419.2 [2 + Na]^+ and m/z 1395.4 [2M − H]^−) spectrum. Analysis of the 1H and 13C NMR data indicated compound 4 consisted of an 8-epiloganic acid skeleton, a caffeoyl group, a cinnamyl group and a glucose moiety fragment. Combined the 1H nMR, 13C nMR and HMBC spectra, the correlations from H-1’ at δ\(_{H}\) 4.72 (1H, d, J = 8.0 Hz) to C-1 at δ\(_{C}\) 96.2 and H-7 at δ\(_{H}\) 4.91 to C-9”′ at δ\(_{C}\) 169.0 deduced the β-D glucopyranosyl unit and the cinnamyl ester group are located to C-1 and C-6 of the aglycone, respectively. The correlation from H-6’ at δ\(_{H}\) 4.54 and 4.42 to C-9” at δ\(_{C}\) 169.1 indicated that the caffeoyl ester group is attached to C-6” of the glucoside. The above information together constructed an agnucastoside C structure of 4. Meanwhile, detailed NMR analysis showed an additional methoxyl group in 4 at δ\(_{H}\) 3.83 (3H, s, OCH\(_{3}\)) and δ\(_{C}\) 56.5. The location of the methoxyl group was at C-3”, confirming from the significant correlation peaks from the methoxyl proton to C-3” in the HMBC spectrum [Figure 2]. The relative configuration of 4 was established by a combination of the 1H NMR spectrum, NOESY experiment and the literature report (Liang et al. 2011). The presence of NOE interactions of H-1, H-7 and H-10 indicated that the hydroxyl group at C-7 should be β-orientation. Thus, the structure of 4 was elucidated as 3”-methoxy-agnucastoside C.

Besides the four new iridoid glycosides, ten known iridoid glycosides were also isolated from this plant. Their structure was identified by comparing the NMR properties with the reported data.

The cytotoxicity on Hela and HeyA8 cell lines in vitro of compounds 1–14 was determined using the microculture tetrazolium (MTT) assay, with taxtol as positive control (Table 1). Among the isolates, only compound 8 showed cytotoxicity against the HeyA8 cell line, with an IC\(_{50}\) value of 36.2 μM. Compounds 4, 5, 8, 12 and 14 showed cytotoxic activities against the Hela cell line with IC\(_{50}\) values of 25.3, 48.1, 17.3, 38.3 and 28.2 μM, respectively. Whereas the other compounds were inactive. Comparing the cytotoxicity results with those of inactive compound (1–3, 6, 7, 9–11 and 13), demonstrated that the agnucastoside C skeleton with an COOH substitution at C-4 plays an important role in the cytotoxicity against Hela cancer
Cell line, and the presence of caffeoyl group and cinnamyl group promotes the cytotoxicity of the iridoid glycosides.

3. Experimental

3.1. General experimental procedures

Optical rotations were measured with a PerkinElmer 341 polarimeter with MeOH as solvent. The UV spectra were performed on a Thermo Scientific Evolution 300 UV–visible spectrophotometer in MeOH. The \(^1\text{H}\) (500 MHz), \(^{13}\text{C}\) (125 MHz) and 2D NMR spectra were recorded on a Bruker DRX-500 instrument using TMS as internal standard. ESI–MS were collected on a MDS SCIEX API 2000 LC/GC/MS instrument. HR–ESI–MS were carried out on a Bruker Bio-TOF-IIQ mass spectrometer. Preparative HPLC was run with a Waters 1525 pump and a Waters 2489 ultraviolet–visible detector using an Xterra® prep MS C18 column (10 um, 7.8 × 150 mm).

3.2. Plant material

The aerial part of \(C. \text{nudiflora}\) was collected from Five Finger Mountain of Hainan Province, People's Republic of China, in April 2013. The plant was identified by BintaoLi of Shenzhen Fairy Lake Botanical garden, Chinese Academy of Sciences. A voucher specimen (No. 20130916) has been deposited in the herbarium of Shenzhen Fairylake Botanical garden, Chinese Academy of Sciences.

3.3. Extraction and isolation

The air-dried material (10 kg) was powdered and extracted three times with 95% EtOH at room temperature to obtain a concentrated extract (860 g), which was suspended in 60% EtOH and partitioned successively with petroleum ether and EtOAc. The EtOAc soluble extract (380 g) was subjected to silica gel column chromatography (CC) using a stepwise gradient of CHCl\(_3\)–MeOH (from 95:5 to 60:40) to afford twenty subfractions Frs. A–T. Fr. H (27.5 g) was chromatographed successively on Sephadex LH-20 CC with the eluent of MeOH two times and preparative RP-C\(_{18}\) HPLC with the stepwise gradient of MeOH–H\(_2\)O (from 50 to 60%) (254 nm, flow rate: 10 mL/min) to yield seven subfractions Frs. H1–7. Fr. H2 (605 mg) was applied to preparative RP-C\(_{18}\) HPLC with the stepwise gradient of MeOH–H\(_2\)O (from 40 to 66%) (254 nm, flow rate: 5 mL/min) to yield compounds 5 (13.0 mg) and 13 (12.3 mg), Fr. H3 (1.36 g) was purified successively by silica gel CC using the eluent of CHCl\(_3\)–MeOH (22:1)

| Compounds | Hela \((\mu \text{M})\) | HeyA8 \((\mu \text{M})\) |
|-----------|-----------------|-----------------|
| 4         | 25.3 ± 2.5      | >50             |
| 5         | 48.1 ± 1.9      | >50             |
| 8         | 17.3 ± 3.0      | 36.2 ± 2.2      |
| 12        | 38.3 ± 4.3      | >50             |
| 13        | 28.2 ± 2.8      | >50             |
| Taxtol    | 1.01 ± 0.10     | 0.98 ± 0.05     |

Compounds 1–3, 6, 7, 9–11 and 13 were inactive for the two cell lines \((IC_{50} > \mu \text{M})\).
and Sephadex LH-20 CC with the eluent of MeOH to give compounds 4 (50.6 mg) and 9 (6.4 mg). Fr. H4 (983 mg) was purified successively by silica gel CC using the eluent of CHCl3–MeOH (10:1) and Sephadex LH-20 CC with the eluent of MeOH to give compound 8 (196.6 mg). Fr. H7 (913 mg) was purified successively by silica gel CC using the eluent of CHCl3–MeOH (10:1) and Sephadex LH-20 CC with the eluent of MeOH to give compound 8 (196.6 mg). Fr. H7 (1.56 g) was purified successively by silica gel CC using the eluent of CHCl3–MeOH (10:1) and Sephadex LH-20 CC with the eluent of MeOH to give compound 8 (196.6 mg). Fr. H7 (1.56 g) was purified successively by preparative RP-C18 HPLC with the stepwise gradient of MeOH–H2O (from 45% to 55%) (254 nm, flow rate: 10 mL/min), silica gel CC using the eluent of CHCl3–MeOH (17:3) and Sephadex LH-20 CC with the eluent of MeOH to give compound 14 (101.6 mg). Fr. G (21.9 g) was subjected to silica gel CC using a stepwise gradient of CHCl3–MeOH (from 10:1 to 8:1) to afford five subfractions Frs. G1–5. Fr. g5 (3.86 g) was chromatographed on Sephadex LH-20 CC with the eluent of MeOH to yield three subfractions Frs. g5-1–3 based on TLC. Fr. g5-2 (875 mg) was subjected to silica gel CC using the eluent of CHCl3–MeOH (10:1) and further purified successively by preparative RP-C18 HPLC with the stepwise gradient of MeOH–H2O (from 40 to 60%) (254 nm, flow rate: 10 mL/min) and Sephadex LH-20 CC with the eluent of MeOH to give compounds 10 (73.8 mg) and 11 (8.4 mg). Fr. g5-3 (1.47 g) was purified successively by preparative RP-C18 HPLC with the stepwise gradient of MeOH–H2O (from 35% to 50%) (254 nm, flow rate: 10 mL/min) and Sephadex LH-20 CC with the eluent of MeOH to give compounds 3 (90.7 mg), 6 (50.6 mg), 7 (89.7 mg) and 12 (30.2 mg). Fr. F (15.6 g) was subjected to silica gel CC using a stepwise gradient of CHCl3–MeOH (from 12:1 to 9:1) to afford five subfractions Frs. F1–F5. Fr. F3 (793 mg) was purified successively by preparative RP-C18 HPLC with the stepwise gradient of MeOH–H2O (from 50 to 65%) (254 nm, flow rate: 5 mL/min) and Sephadex LH-20 CC with the eluent of MeOH to give compounds 1 (15.3 mg) and 2 (9.8 mg).

3.3.1. 5”-methoxy-ampicoside (1)
White amorphous powder, [α]D20 = −160° (c 1, MeOH); UV λmax (MeOH) nm (log ε): 251.3 (3.58) and 278.8 (3.84); ESI-MS: m/z 541.8 [M − H]− and m/z 1083.3 [2M − H]−; HR-ESI-MS: m/z 565.1520 [M + Na]⁺ (Calcd for C24H30NaO14: 565.1528, erro +1.4 ppm).

1H NMR(DMSO-d6, 500 MHz) δ: 5.12 (1H, d, J = 9.5 Hz, H-1), 6.43 (1H, dd, J = 6.0 Hz, H-3), 4.99 (1H, dd, J = 5.5 Hz, H-4), 2.61 (1H, m), 2.07 (1H, m), 3.17 (1H, m), 2.47 (1H, t, J = 8.8 Hz, H-7), 3.74 (1H, dd, J = 13.6 Hz, H-10a), 3.91 (1H, dd, J = 13.5 Hz, H-10b), 5.07 (1H, brd, J = 8.0 Hz, H-6), 3.71 (1H, brs, H-7), 2.47 (1H, t, J = 8.8 Hz, H-9), 3.80 (1H, d, J = 8.0 Hz, H-6), 4.62 (1H, d, J = 8.0 Hz, glu-H-1'), 3.04 (1H, m, glu-H-2', 4'), 3.18 (1H, m, glu-H-3', 5'), 3.43 (1H, dd, J = 13.7 Hz, H-10a), 3.69 (1H, dd, J = 13.7 Hz, H-10b), 7.25 (2H, s, H-2', 6'), 3.83 (6H, s, 3',5'-OCH3).

13C NMR(DMSO-d6, 125 MHz) δ: 93.4 (C-1), 141.5 (C-3), 102.3 (C-4), 35.6 (C-5), 80.3 (C-6), 58.7 (C-7), 66.4 (C-8), 42.3 (C-9), 58.9 (C-10), 98.3 (glu-C-1'), 73.9 (glu-C-2'), 76.9 (glu-C-3'), 70.7 (glu-C-4'), 77.9 (glu-C-5'), 61.9 (glu-C-6'), 119.2 (C-1”), 107.4 (C-2”, 6”), 148.0 (C-3”, 5”), 141.5 (C-4”), 166.1 (C-7”), 56.6 (3’, 5’-OCH3).

3.3.2. 6”-O-trans-cafeoylcatapalol (2)
White amorphous powder, [α]D20 = −87° (c 1, MeOH); UV λmax (MeOH) nm (log ε): 244.6 (3.69) and 329.9 (4.05); ESI–MS: m/z 547.3 [M + Na]⁺, 1071.4 [2M + Na]⁺, 523.9 [M − H]− and 1047.5 [2M − H]−; HR-ESI-MS: m/z 547.1435 [M + Na]⁺ (Calcd for C24H28NaO13: 547.1422, erro −2.3 ppm).

1H NMR(CD3OD, 400 MHz) δ: 4.87 (1H, d, J = 10.0 Hz, H-1), 6.33 (1H, dd, J = 6.0 Hz, H-3), 5.04 (1H, t, J = 5.2 Hz, H-4), 2.24 (1H, ddd, J = 12.0 Hz, H-5), 3.80 (1H, d, J = 8.0 Hz, H-6), 3.43 (1H, m, H-7), 2.55 (1H, dd, J = 9.6 Hz, H-9), 3.66 (1H, d, J = 13.2 Hz, H-10a), 4.17 (1H, d,
3.3.3. 6″-O-feruloylcatalpol (3)

White amorphous powder, \([\alpha]_D^{20} = -85^\circ\) (c 5, MeOH); UV \(\lambda_{max}\) (MeOH) nm (log \(\varepsilon\)): 236.4 (3.98) and 326.7 (4.35); ESI-MS: 561.2 [M + Na]^+ and m/z 1075.3 [2M – H]^-; HR-ESI-MS: m/z 561.1596 [M + Na]^+ (Calcld for C_{35}H_{38}NaO_{14}Na: 561.1579, erro – 3.1 ppm).

\(^1\)H NMR (CD_3OD, 500 MHz) \(\delta\): 4.88 (1H, d, J = 9.5 Hz, H-1), 6.33 (1H, dd, J = 6.01 Hz, H-3), 4.99 (1H, t, J = 5.6 Hz, H-4), 2.25 (1H, ddd, J = 8.56.01.5 Hz, H-5), 3.81 (1H, brd, J = 8.0 Hz, H-6), 3.57 (1H, m, H-7), 2.56 (1H, dd, J = 8.01.6 Hz, H-9), 3.68 (1H, d, J = 13.0 Hz, H-10a), 4.18 (1H, d, J = 13.0 Hz, H-10b), 4.81 (1H, d, J = 7.8 Hz, glu-H-1′), 3.33 (1H, m, glu-H-2′), 3.44 (1H, m, glu-H-3′), 3.47 (1H, m, glu-H-4′), 3.58 (1H, dd, J = 12.0 Hz, H-5′), 4.47 (1H, dd, J = 12.02 Hz, H-6′a), 3.50 (1H, d, J = 12.06 Hz, H-6′b), 7.20 (1H, d, J = 2.0 Hz, H-2′), 6.84 (1H, d, J = 8.0 Hz, H-5″), 7.10 (1H, dd, J = 8.02 Hz, H-6″), 7.64 (1H, d, J = 16.0 Hz, H-7″), 6.40 (1H, d, J = 16.0 Hz, H-8″), 3.90 (3H, s, OCH_3); \(^13\)C NMR (CD_3OD, 125 MHz) \(\delta\): 95.3 (C-1), 141.8 (C-3), 104.0 (C-4), 39.1 (C-5), 79.7 (C-6), 62.4 (C-7), 66.1 (C-8), 43.4 (C-9), 61.9 (C-10), 99.8 (glu-C-1′), 74.7 (glu-C-2′), 77.4 (glu-C-3′), 71.6 (glu-C-4′), 75.9 (glu-C-5′), 63.9 (glu-C-6′), 127.6 (C-1″), 111.7 (C-2″), 149.4 (C-3″), 150.7 (C-4″), 116.5 (C-5″), 124.2 (C-6″), 147.1 (C-7″), 115.2 (C-8″), 168.9 (C-9″), 56.5 (3″-OCH_3).

3.3.4. 3″-methoxy-agnucastoside C (4)

White amorphous powder, \([\alpha]_D^{20} = -27^\circ\) (c 1, MeOH); UV \(\lambda_{max}\) (MeOH) nm (log \(\varepsilon\)): 230.7 (4.01) and 316.2 (4.22); ESI-MS: m/z 699.2 [M + H]^+, m/z 721.2 [M + Na]^+, m/z 1419.2 [2M + Na]^+ and m/z 1395.4 [2M – H]^-; HR-ESI-MS: m/z 721.2089 [M + Na]^+ (Calcld for C_{38}H_{38}NaO_{15}: 721.2103, erro +1.9 ppm).

\(^1\)H NMR(CD_3OD,500 MHz) \(\delta\): 5.29 (1H, d, J = 5.6 Hz, H-1), 7.36 (1H, s, H-3), 3.06 (1H, dd, J = 9.680 Hz, H-5), 2.15 (1H, m, H-6a), 1.92 (1H, dd, J = 8.580 Hz, H-6b), 4.91 (1H, m, H-7), 2.43 (1H, m, H-8), 2.87 (1H, t, J = 5.046 Hz, H-9), 1.09 (1H, d, J = 7.0 Hz, H-10), 4.72 (1H, d, J = 8.0 Hz, glu-H-1′), 3.26 (1H, m, glu-H-2′), 3.41 (1H, m, glu-H-3′), 3.38 (1H, m, glu-H-4′), 3.56 (1H, m, glu-H-5′), 4.54 (1H, dd, J = 12.02 Hz, glu-H-6′a), 4.42 (1H, dd, J = 12.065 Hz, glu-H-6′b), 7.19 (1H, d, J = 2.0 Hz, H-2″), 6.76 (1H, d, J = 8.0 Hz, H-5″), 7.03 (1H, dd, J = 8.520 Hz, H-6″), 7.64 (1H, d, J = 16.0 Hz, H-7″), 6.41 (1H, d, J = 16.0 Hz, H-8″), 3.83 (3H, s, OCH_3), 7.42 (1H, d, J = 8.5 Hz, H-2″″, 6″″), 6.79 (1H, d, J = 8.5 Hz, H-3″″, 5″″), 7.55 (1H, d, J = 16.0 Hz, H-7″″), 6.28 (1H, d, J = 16.0 Hz, H-8″″); \(^13\)C NMR(CD_3OD,125 MHz) \(\delta\): 96.2 (C-1), 151.8 (C-3), 116.5 (C-4), 33.1 (C-5), 39.8 (C-6), 82.3 (C-7), 43.4 (C-8), 43.6 (C-9), 145.7 (C-10), 169.1 (C-11), 100.0 (glu-C-1′), 74.9 (glu-C-2′), 77.9 (glu-C-3′), 71.9 (glu-C-4′), 75.8 (glu-C-5′), 64.1 (glu-C-6′), 127.7 (C-1″), 111.7 (C-2″), 149.4 (C-3″), 150.6 (C-4″), 116.4 (C-5″), 124.4 (C-6″), 147.3 (C-7″), 115.3 (C-8″), 169.1 (C-9″), 56.5 (3″-OCH_3), 127.2 (C-1″″), 131.2 (C-2″″), 116.8 (C-3″″), 161.2 (C-4″″), 116.8 (C-5″″), 131.2 (C-6″″), 146.4 (C-7″″), 115.5 (C-8″″), 169.0 (C-9″″).
3.4. GC analysis of the sugar moieties in 1–4

Compounds 1–4 (each 4 mg) in 1 M HCl (5 mL, dioxane–H₂O 1:1, v/v) were heated at 95 °C for 6 h, respectively. The reaction mixtures were evaporated in vacuo. The residues were dissolved in water and then extracted with CHCl₃ for three times, respectively. After evaporation the aqueous phases to dryness in vacuo, the residues were dissolved in pyridine (5 mL) and 1-(trimethylsilyl)-imidazole (0.5 mL) at room temperature for 30 min. The reaction mixtures were dried with a stream of N₂. The residues were partitioned between CHCl₃ and H₂O. The organic layers were subjected to GC analysis using an L-Chirasil-Val column (0.32 mm × 25 m) [14]. Temperature of the injector and detector was 200 °C. A temperature gradient system was used for the oven; the initial temperature was maintained at 100 °C for 1 min and then increased up to 180 °C at a rate of 5 °C/min. Peaks of the hydrolysate of 1–4 were, respectively, detected at 14.56, 14.57, 14.55 and 14.57 min, suggested that all the sugar moieties of 1–4 are d-glucose. Retention times for authentic samples of d-glucose (Sigma-Aldrich, St. Louis, MO, USA) after being treated in the same manner with 1-(trimethylsilyl)-imidazole in pyridine were detected at 14.56 min.

3.5. Cytotoxicity assay

Hela and HeyA8 cell lines (purchased from Shanghai Institutes for Biological Science, Chinese Academy of Sciences) were cultured in DMEM (Gibco BRL, Grand Island, NY, USA) supplemented with 10% foetal bovine serum (FBS) (Gibco BRL, Grand Island, NY, USA). The cells were cultured at 37 °C in a humidified atmosphere containing 5% CO₂. Cytotoxicity was measured by a MTT assay with minor modification to the reference procedure (Mosmann 1983). Briefly, cells were seeded in 96-well microculture plates and cultured for 24 h and then various concentration of tested drug was added, with taxol (Sigma) as positive control. After the incubation for another 48 h, 20 μL of MTT (5 mg/mL) was added to each well, and the incubation continued for 4 h at 37 °C. After 4 h, 100 μL of DMSO was added to each well to dissolve the formazan crystals of the viable cells. The plates were read at a wavelength of 590 nm using microplate reader. IC₅₀ values were calculated using SPSS 17.0 statistical analysis software.

Disclosure statement

No potential conflict of interest was reported by the authors.

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