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Highly regioselective glycosylation of xylosyl-containing taxanes by *Enterobacter cloaceae*

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ABSTRACT

Three new diglycoside taxanes (**4**–**6**) containing an additional α-D-glucose unit were obtained via *Enterobacter cloaceae*-mediated, highly regioselective glycosylations at OH-4 of the xylosyl moieties in 7-xylosyl taxanes (**1**–**3**). Their structures were determined on the basis of extensive spectroscopic analysis and chemical reactivity. This is the first report of an enzymatic elongation of the saccharide chain in taxanes, and the glycosylation reaction is the first described example of a transformation effected by *E. cloaceae*.

1. Introduction

Glycosylation is one of the most common and important transformations in biological systems, and the resulting glycoconjugates have diverse functions [1]. Most of the enzymes that mediate glycosylation are glycosyltransferases (GTs), which can also be used for the glycosylation of foreign substrates, such as small molecules of natural products. Given the fact that glycosides are capable of accessing a wide range of unique chemical space, sugars attached to natural products can clearly complement and expand the chemical diversity of the molecules. Glycosylation can also dramatically influence the pharmacological and pharmacokinetic properties of drugs, such as solubility, distribution, and metabolic stability, among others. Furthermore, it has been demonstrated that the cellular uptake of many active components can be remarkably enhanced following glycosylation, due to active absorption mediated by glucose transport systems [1–4]. Thus, the glycosylation of natural products has emerged as a viable strategy to produce bioactive compounds with improved activity. However, glycosylation, especially regioselective glycosylation of natural products remains an onerous task for organic chemists, often due to the presence of multiple reactive hydroxyl groups. The use of biocatalysts such as microbes has opened up new opportunities for modification because of their high selectivity, simplicity, and mild reaction conditions; in addition, their use is environmentally friendly.

As part of an on-going investigation into the biotransformations of taxanes [5–9], three xylosyl-containing taxanes, 7-xylosyl-10-deacetyltaxol (7-XDT, **1**), 7-xylosyl-10-deacetylcephalomannine (7-XDC, **2**), and 7-xylosyl-10-deacetyltaxol C (7-XDTC, **3**), were used as substrates for various microorganisms with some interesting results. For example, we previously reported the effective microbial hydrolysis of 7-XDT to 10-deacetyltaxol by a new bacterial strain isolated from soil [8]. Herein, we report the transformations of these three xylosyl-containing taxanes by *Enterobacter cloaceae* CGMCC 1.181 (purchased from China General Microbiological Culture Collection Center, Beijing, China), specifically their regioselective glycosylation and the structural elucidation of the corresponding glycosylated metabolites (Fig. 1).

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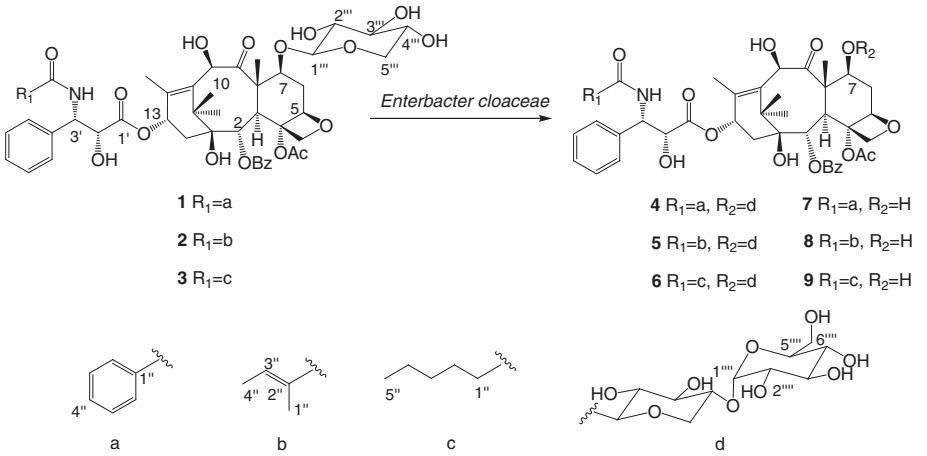

Fig. 1. The structures of xylosyl-containing taxanes (**1**–**3**) and their metabolites (**4**–**9**) from *Enterobacter cloaceae* CGMCC 1.181.

2. Results and discussion

Based on the HPLC-DAD-MS analyses, the biotransformation of these three xylosyl-containing taxanes (**1**–**3**) by the strain *E. cloaceae* CGMCC 1.181 exhibited the similar patterns of metabolites in HPLC spectra, in which the corresponding hydrolysis products, 10-deacetyltaxol (10-DT, **7**), 10-deacetylcephalomannine (10-DC, **8**), and 10-deacetyltaxol C (10-DTC, **9**) were detected in line with expectations respectively. Interestingly, there were also three additional metabolites with identical characteristic UV spectra to its substrates, which were eluted a little earlier than the substrates (see Supplementary Material, Fig. S1). And then, the strain *E. cloaceae* CGMCC 1.181 was subsequently cultured for the further preparative biotransformation of substrates **1**–**3**. After a two-stage fermentation procedure and the use of various preparative chromatographic techniques, six metabolites were obtained from the transformation of **1**–**3** by *E. cloaceae*. On the basis of the physical and spectroscopy data, the structures of these metabolites were assigned as 7-*O*-[α-D-glucopyranosyl-(1→4)-β-D-xylopyranosyl]-10-deacetyltaxol (**4**) and 10-deacetyltaxol (**7**), 7-*O*-[α-D-glucopyranosyl-(1→4)-β-D-xylopyranosyl]-10-deacetylcephalomannine (**5**), 10-deacetylcephalomannine (**8**), 7-*O*-[α-D-glucopyranosyl-(1→4)-β-D-xylopyranosyl]-10-deacetyltaxol C (**6**), and 10-deacetyltaxol C (**9**). In addition to the occurrence of the site-specific hydrolysis reaction, similar to that observed with *Enterobacter* sp. CGMCC 2487 [8], selective glycosylation also occurred to yield three novel compounds containing a diglycosyl group.

Mass spectroscopy of compound **4** exhibited a signal corresponding to the molecular formula $C_{56}H_{67}NO_{22}$, suggesting 24 degrees of unsaturation. The molecular mass of **4** was found to be 162 amu higher than that of **1**, which indicates that an additional hexosyl group had been attached to molecule **1** via glycosylation. Compared with the ^{1}H and ^{13}C NMR spectra of **1**, the NMR spectra of **4** contained signals corresponding to the taxane skeleton of **1** and a xylosyl moiety. The HMBC spectrum displayed strong correlations between the signal at δ_H 82.6 (C-7) and the anomeric hydrogen of the xylosyl group at δ_H 4.58 (H-1′′′, d, J= 7.8 Hz,), which suggests that the xylosyl group is still attached to OH-7. The ^{13}C NMR spectrum of **4** contained an additional signal at δ_C 102.4 indicating the presence of an anomeric carbon and five additional signals between 60 and 80 ppm, attributed to oxygen-bearing carbons of the sugar moiety. No significant differences were observed in the chemical shifts of C-1, C-10 and C-2′ of **4** compared with **1**, supporting the deduction that **4** is a glycosylated derivative of **1** containing an additional hexosyl group attached to the parent xylosyl moiety. To establish the location of the glycosylation linkage, the ^{13}C NMR signals corresponding to the xylosyl moiety of **4** were assigned from the HMQC and HMBC spectra. Upon comparison of the chemical shifts of **4** and **1**, a downfield signal corresponding to glycosylation at δ_C 76.9 (C-4′′′ of the xylosyl moiety) in **4** and an upfield shift of the δ_C 65.3 (C-5′′′) signal was observed. Thus, the glycosylation linkage occurred on the OH-4 of the xylosyl moiety. The GC-MS analysis and comparison to standards was performed with the compound obtained after hydrolysis, revealing that **4** contained only a D-xylose unit and a D-glucose unit, as shown by agreement with the standards [2,3,4,5,-tetra-*O*-acetyl-D-xylononitrile (t_R 12.099 min), and 2,3,4,5,6-penta-*O*-acetyl-D-gluconitrile (t_R 15.043 min)] (Figs. S4 and S5). The ^{1}H NMR spectrum displayed a signal at δ_H 5.61 (1H, d, J= 4.2 Hz) corresponding to the anomeric hydrogen of the glucose unit, and the small coupling constant indicated that the second sugar unit was α-D-glucose containing a β-anomeric hydrogen (H-1′′′′) *syn*-oriented to the neighboring hydrogen (H-2′′′′). Accordingly, the identity of **4** was determined to be 7-*O*-[α-D-glucopyranosyl-(1→4)-β-D-xylopyranosyl]-10-deacetyltaxol.

The HRESIMS, ^{1}H and ^{13}C NMR spectra of **5** showed that it was composed of **2** and an additional glucose unit by the molecular formula $C_{54}H_{69}NO_{22}$. The ^{13}C NMR spectrum displayed signals corresponding to the anomeric carbon (δ_C 102.3), additional tertiary carbons (δ_C 74.1, 75.4, 71.9, 74.9), and a terminal secondary carbon (δ_C 62.9) identical to those of **4**. Similarly, **2** had also been converted to the corresponding metabolite by glycosylation. The similarity between the ^{1}H and ^{13}C NMR signals of the two sugar units in **4** and **5** indicated the presence of a 7-*O*-α-D-glucopyranosyl-(1→4)-

β-D-xylopyranosyl group in **5**. Based on the further analysis of the HMQC and HMBC spectra, the identity of **5** was confirmed to be 7-*O*-[α-D-glucopyranosyl-(1→4)-β-D-xylopyranosyl]-10-deacetylcephalomannine.

The molecular formula of **6** was determined to be $C_{55}H_{74}NO_{22}$ from analysis of the HR-ESIMS spectrum, containing a peak 162 amu higher than the molecular mass of **3**. The major differences between the ^{1}H and ^{13}C NMR spectra of **6** and **3** appeared in the signals resulting from the presence of an additional sugar moiety similar to **4** and **5**. The additional sugar unit was determined to be an α-D-glucose linked to the C-4‴ of the xylosyl group from the ^{1}H NMR, HMQC and HMBC spectral data. Accordingly, **6** was identified as 7-*O*-[α-D-glucopyranosyl-(1→4)-β-D-xylopyranosyl]-10-deacetyltaxol C.

Metabolites **2–9** together with **1** and the positive control paclitaxel, were tested for their cytotoxicity against five human cancer cell lines. The results are given in Table 1. As listed, these compounds showed less potent activities than paclitaxel. Obviously, sugar moiety (monosaccharide and disaccharide) at C-7 decreased the activity of these taxanes, and there was no difference in the influence on the activity between monosaccharide and disaccharide taxanes.

In summary, this report describes the selective glycosylation and dexylosylation of 7-xylosyl taxanes (**1**–**3**) by *E. cloaceae* CGMCC 1.181. Interestingly, the two reverse reactions simultaneously occur during the same bio-process. This discovery produces a puzzle to be investigated regarding whether the glycosylation reaction occurs only following hydrolysis to install a new diglycosyl group on C-7 of the α-D-glucopyranosyl-(1→4)-β-D-xylose or this enzyme simply attaches glucosyl units directly onto its substrates. The fact that *E. cloaceae* CGMCC 1.181 possesses the capacity to selectively remove the xylosyl moiety from 7-xylosyl taxanes, similar to the reported *Enterobacter* sp. CGMCC 2487 isolated from soil, suggests the existence of a similar xylosylase arising from two species in the same genus. Selective glycosylation was observed only in the transformation mediated by *E. cloaceae* CGMCC 1.181, highlighting the differences that exist between the two species. In addition, both enzymes could accept three structurally different substrates, implying some substrate tolerance of the two enzymes. Most importantly, to the best of our knowledge, this is the first report of an enzymatic synthesis of the disaccharide taxanes and is the first example of an enzymatic glycosylation of a natural product by *Enterobacter* species.

Table 1
Cytotoxicity of metabolites **1–9** against human cancer cells.

Compounds	IC_{50} (μM)				
	HCT-8	Bel-7402	BGC-823	A549	A2780
1	>10	6.80	0.35	>10	0.50
2	>10	>10	1.37	>10	0.55
3	>10	>10	0.91	>10	0.48
4	>10	>10	0.49	>10	0.47
5	>10	>10	0.63	>10	0.58
6	>10	>10	0.99	>10	1.0
7	6.68	1.13	0.1	>10	0.05
8	4.19	2.48	0.06	>10	0.03
9	8.12	1.09	0.02	>10	0.06
Paclitaxel	0.037	0.1	0.007	0.019	0.0063

3. Experimental

3.1. General experimental procedures

The ^{1}H- and ^{13}C-NMR spectra were recorded on Bruker ARX-400 spectrometers using $CDCl_3$ as solvent and internal reference. ESIMS spectra were obtained using a VG ZabSpec mass spectrometer. Analytical HPLC was carried out on an Agilent 1200 with a BDS HYDERSIL column (C_{18}, 5 μm, 250 mm × 4.6 mm i.d., the flow-rate was 1 mL/min), the UV detector was set at 230 nm, and the column was operated at 30 °C. HPLC analysis was achieved with a two-pump gradient program for pump A (solution A: MeCN) and pump B (solution B: water) as follows: 30% solution A, ramped to 38% solution A within 12 min, then to 52% solution A until 30 min; held at 52% solution A for 2 min, then reset to 30% solution A until 40 min for equilibrating the column and stabilizing the baseline before the next injection. Semi-preparative reverse-phase HPLC was performed on a Shimadzu LC-6AD instrument with a YMC-Pack ODS-A (5 μm, 250 mm × 10 mm i.d., the flow-rate was 2 mL/min) and a Shimadzu RID-10A detector. Si gel (200–300 mesh) was used for flash column chromatography. Analytical TLC was carried out on Si gel GF254 plates (Qingdao Oceanic Chemicals, China), and the visualization of TLC plates was performed by spraying with 5% H_2SO_4 in EtOH followed by heating at 105 °C. A LCQ Fleet ion trap mass spectrometer (ThermoFisher, USA) was connected to the Agilent 1200 HPLC instrument via an ESI interface. The LC effluent was introduced into the ESI source in a post-column splitting ratio of 10:1. Ultrahigh-purity helium (He) was used as the collision gas and high-purity nitrogen (N_2) as the nebulizing gas. The optimized parameters in the positive ion mode were as follows: ion spray voltage, 4.5 KV; capillary temperature, 325 °C; sheath gas (N_2), 6 volume/min. For full scan MS analysis, the spectra were recorded in the range of *m/z* 750−1050. HPLC grade acetonitrile (Fisher) and ultra-pure water were used for all analyses. GC-MS was performed on a Thermo-Electron instrument coupled to a Thermo-Electron spectrometer. GC conditions: DB-5MS (5% phenyl)-methylpolysiloxane colum (30 m × 0.25 mm, film thickness 0.1 m); He linear velocity, 30 cm/s; 50 °C isothermal for 4 min, linear gradient to 300 at 40 °C/min; final temperature hold, 20 min. MS conditions: ionization energy, 70 eV; ion source temperature, 250 °C; interface temperature, 270 °C; mass range, 45−600 amu. AR grade methanol, ethyl acetate, dichloromethane were purchased from Beijing Chemical Corporation (Beijing, China). The conversion rates of products were calculated by the molar ratio of obtained amount of products to the added amount of substrates.

3.2. Substrates and organism

The mixture of substrates, 7β-D-xylosyl-10-deacetyltaxol (**1**), 7β-D-xylosyl-10-deacetylcephalomannine (**2**) and 7β-D-xylosyl-10-deacetyltaxol C (**3**) were gifts from Guilin Hui'ang Biochemistry Medicine Industry Co. Ltd., Guilin, China. The bacterial strain *E. cloaceae* CGMCC 1.181 was purchased from

China General Microbiological Culture Collection Center. The strain was maintained on slants containing wheat bran medium [50 g of wheat bran, 2.0 g of K_2HPO_4, 4.0 g of $(NH_4)_2SO_4$, and 1000 mL of distilled water].

3.3. Preparative biotransformation

A two-stage fermentation procedure was used. The bran medium contained 50 g of wheat bran (boiled with 800 ml of H_2O for 30 min, and then filtered for use), 2 g of K_2HPO_4, 4 g of $(NH_4)_2SO_4$, and 0.6 g of oat xylan in 1 L of distilled water, adjusted to pH 6.0 before autoclaving. Eight milliliters of two-day-old seeded *E. cloaceae* strain was inoculated into ten 1 L-flasks with 400 mL medium in each flask. After cultivation for 2 days at 120 rpm and 26 ± 2 °C, the bacterial cells were harvested through centrifugation at 7000 rpm for 20 min and were then re-suspended with 1 L of 0.5 M phosphate buffer (pH 6.0) and distributed into 5 flasks. Next, the mixture of 7-XDT (**1**, 230 mg), 7-XDC (**2**, 100 mg) and 7-XDTC (**3**, 150 mg) dissolved in an appropriate volume of DMF were added into the above buffer solution containing the bacterial cells. After incubating for 7 days at 120 rpm and 26 ± 2 °C, the cultures were pooled and centrifuged. The supernatant was extracted with ethyl acetate to obtain the crude extracts. The crude extracts were then separated with a gradient of dichloromethane/methanol on a silica gel chromatography column and subsequently purified by reverse-phase semi-preparative HPLC. Six compounds were obtained with an eluent of MeCN/H_2O (35/65, v/v) at a flow rate of 4 mL/min: **4** (8.0 mg, t_R = 17.74 min, 3.0%), **7** (15.0 mg, t_R = 23.56 min, 7.6%), **5** (6.1 mg, t_R = 15.89 min, 5.2%), **8** (8.0 mg, t_R = 21.55 min, 9.3%); **6** (8.1 mg, t_R = 20.72 min, 4.6%), **9** (9.1 mg, t_R = 27.04 min, 7.0%). The recovered substrates were 110 mg (**1**, 47.8%), 50 (**2**, 50.0%), and 70 mg (**3**, 46.7%).

3.3.1. 7-O-[α-D-glucopyranosyl-(1→4)-β-D-xylopyranosyl]-10-deacetyltaxol (4)

White powder (mp > 200 °C); $[\alpha]^{25}_D$ +11 (*c* 0.235, MeOH); IR ν_{max} (micro): 3429, 3260, 2958, 1745, 1658, 1627, 1504, 1280, 1228, 1123, 711 cm^{-1}; ^1H NMR (600 MHz, pyridine-d_5) δ 6.25 (1H, d, *J* = 6.6 Hz, H-2), 4.42 (1H, dd, *J* = 11.4, 4.8 Hz, H-3), 5.24 (1H, d, *J* = 9.6 Hz, H-5), 3.02 (1H, t, *J* = 13.8 Hz, H-6a), 2.35 (1H, t, *J* = 13.8 Hz, H-6b), 4.75 (1H, dd, *J* = 10.2, 7.2 Hz, H-7), 6.10 (1H, s, H-10), 6.79 (1H, t, *J* = 9.0 Hz, H-13), 2.85 (2H, m, H-14), 1.63 (3H, s, H-16), 2.29 (3H, s, H-17), 1.47 (3H, s, H-18), 2.20 (3H, s, H-19), 4.59 (1H, d, *J* = 8.4 Hz, H-20a), 4.49 (1H, m, H-20b), 5.37 (1H, s, H-2′), 6.42 (1H, dd, *J* = 8.4, 4.2 Hz, H-3′), 7.33 (2H, d, *J* = 7.8 Hz, 3′-C_6H_5 moiety-H_o), 7.82 (2H, t, *J* = 7.8 Hz, 3′-C_6H_5 moiety-H_m), 7.26 (1H, t, *J* = 7.8 Hz, 3′-C_6H_5 moiety-H_p), 2.52 (3H, s, 4-$OCOCH_3$), 8.35 (2H, d, *J* = 7.2 Hz, 2-$OCOC_6H_5$ moiety-H_o), 7.45 (2H, t, *J* = 7.2 Hz, 2-$OCOC_6H_5$ moiety-H_m), 7.51 (1H, t, *J* = 7.2 Hz, 2-$OCOC_6H_5$ moiety-H_p), 9.42 (1H, d, *J* = 8.4 Hz, NH), 8.35 (2H, d, *J* = 7.2 Hz, H-2″, 6″), 7.26 (2H, t, *J* = 7.2 Hz, H-3″, 5″), 7.37 (1H, t, *J* = 7.2 Hz, H-4″), 4.58 (1H, d, *J* = 7.8 Hz, 7-xylosyl moiety, H-1‴), 3.95 (1H, m, H-2‴), 3.72 (1H, t, *J* = 7.8 Hz, H-3‴), 3.95 (1H, m, H-4‴), 4.42 (1H, d, *J* = 6.6 Hz, H-5‴a), 3.42 (1H, t, *J* = 8.4 Hz, H-5‴b), 5.61 (1H, d, *J* = 4.2 Hz, Glc-moiety, H-1⁗), 4.08 (1H, dd, *J* = 9.6, 3.6 Hz, H-2⁗), 4.50 (1H, d, *J* = 9.6 Hz, H-3⁗), 4.12 (1H, t, *J* = 9.0 Hz, H-4⁗), 4.34 (1H, m, H-5⁗), 4.50 (1H, m, H-6⁗a), 4.34 (1H, m, H-6⁗b); ^{13}C NMR (150 MHz, pyridine-d_5) δ 78.2 (s, C-1), 76.0 (d, C-2), 47.7 (d, C-3), 81.6 (s, C-4), 84.6 (d, C-5), 36.3 (t, C-6), 82.6 (d, C-7), 57.2 (s, C-8), 210.2 (s, C-9), 75.8 (d, C-10), 137.7 (s, C-11), 137.9 (s, C-12), 72.0 (d, C-13), 36.8 (t, C-14), 44.3 (s, C-15), 21.8 (q, C-16), 14.5 (q, C-17), 27.2 (q, C-18), 11.4 (q, C-19), 76.7 (t, C-20), 174.2 (s, C-1′), 74.6 (d, C-2′), 56.9 (d, C-3′), 140.8 (s, 3′-Ph-C_q), 128.6 (d, 3′-Ph-C_o), 128.1 (d, 3′-Ph-C_m), 127.7 (d, 3′-Ph-C_p), 170.7 (s, 4-$OCOCH_3$), 22.8 (q, 4-$OCOCH_3$), 166.4 (s, 2-$OCOC_6H_5$), 131.2 (s, 2-$OCOC_6H_5$-C_q), 130.4 (d, 2-$OCOC_6H_5$-C_o), 128.9 (d, 2-$OCOC_6H_5$-C_m), 133.4 (d, 2-$OCOC_6H_5$-C_p), 168.0 (s, CONH), 131.5 (s, C-1″), 128.1 (d, C-2″, 6″), 128.0 (d, C-3″, 5″), 128.6 (d, C-4″), 106.5 (d, C-1‴), 79.7 (d, C-2‴), 74.3 (d, C-3‴), 76.9 (d, C-4‴), 65.3 (t, C-5‴), 102.4 (d, C-1⁗), 74.1 (d, C-2⁗), 75.4 (d, C-3⁗), 71.9 (d, C-4⁗), 74.9 (d, C-5⁗), 62.9 (t, C-6⁗); (+)-HR-ESIMS *m/z* 1106.4174 $[M+H]^+$ ($C_{56}H_{68}NO_{22}$, calcd for 1106.4227).

3.3.2. 7-O-[α-D-glucopyranosyl-(1→4)-β-D-xylopyranosyl]-10-deacetylcephalomannine (5)

White powder (mp > 200 °C); $[\alpha]^{25}_D$ +9.5 (*c* 0.2, MeOH); IR ν_{max} (micro): 3400, 2928, 1723, 1658, 1273, 1248, 711 cm^{-1}; ^1H NMR (600 MHz, pyridine-d_5) δ 6.26 (1H, m, H-2), 4.42 (1H, dd, *J* = 11.4, 4.8 Hz, H-3), 5.24 (1H, d, *J* = 10.5 Hz, H-5), 3.01 (1H, m, H-6a), 2.35 (1H, m, H-6b), 4.76 (1H, dd, *J* = 10.8, 6.8 Hz, H-7), 6.10 (1H, s, H-10), 6.80 (1H, t, *J* = 8.4 Hz, H-13), 2.85 (2H, m, H-14), 1.64 (3H, s, H-16), 2.30 (3H, s, H-17), 1.50 (3H, s, H-18), 2.21 (3H, s, H-19), 4.59 (1H, d, *J* = 9.0 Hz, H-20a), 4.50 (1H, m, H-20b), 5.30 (1H, s, H-2′), 6.26 (1H, m, H-3′), 7.35 (2H, d, *J* = 7.8 Hz, 3′-C_6H_5 moiety-H_o), 7.78 (2H, t, *J* = 7.8 Hz, 3′-C_6H_5 moiety-H_m), 7.26 (1H, t, *J* = 7.8 Hz, 3′-C_6H_5 moiety-H_p), 2.54 (3H, s, 4-$OCOCH_3$), 8.35 (2H, d, *J* = 7.2 Hz, 2-$OCOC_6H_5$ moiety-H_o), 7.43 (2H, t, *J* = 7.2 Hz, 2-$OCOC_6H_5$ moiety-H_m), 7.50 (1H, t, *J* = 7.2 Hz, 2-$OCOC_6H_5$ moiety-H_p), 8.49 (1H, d, *J* = 8.4 Hz, NH), 1.80 (3H, s, H-1″), 6.55 (1H, m, H-3″), 1.42 (3H, d, *J* = 6.6 Hz, H-4″), 4.58 (1H, d, *J* = 8.4 Hz, 7-xylosyl moiety, H-1‴), 3.95 (1H, m, H-2‴), 3.72 (1H, t, *J* = 7.2 Hz, H-3‴), 3.95 (1H, m, H-4‴), 4.42 (1H, m, H-5‴a), 3.42 (1H, t, *J* = 10.2 Hz, H-5‴b), 5.62 (1H, d, *J* = 4.2 Hz, Glc-moiety, H-1⁗), 4.09 (1H, dd, *J* = 10.2, 3.6 Hz, H-2⁗), 4.50 (1H, d, *J* = 10.8 Hz, H-3⁗), 4.12 (1H, t, *J* = 9.0 Hz, H-4⁗), 4.35 (1H, m, H-5⁗), 4.50 (1H, m, H-6⁗a), 4.35 (1H, m, H-6⁗b); ^{13}C NMR (150 MHz, pyridine-d_5) δ 78.2 (s, C-1), 76.0 (d, C-2), 47.7 (d, C-3), 81.5 (s, C-4), 84.6 (d, C-5), 36.3 (t, C-6), 82.4 (d, C-7), 57.2 (s, C-8), 210.2 (s, C-9), 75.8 (d, C-10), 137.7 (s, C-11), 138.0 (s, C-12), 72.0 (d, C-13), 36.8 (t, C-14), 44.3 (s, C-15), 21.8 (q, C-16), 14.5 (q, C-17), 27.2 (q, C-18), 11.4 (q, C-19), 76.7 (t, C-20), 174.2 (s, C-1′), 74.6 (d, C-2′), 56.3 (d, C-3′), 140.8 (s, 3′-Ph-C_q), 128.8 (d, 3′-Ph-C_o), 128.0 (d, 3′-Ph-C_m), 127.7 (d, 3′-Ph-C_p), 170.7 (s, 4-$OCOCH_3$), 22.8 (q, 4-$OCOCH_3$), 166.4 (s, 2-$OCOC_6H_5$), 131.1 (s, 2-$OCOC_6H_5$-C_q), 130.4 (d, 2-$OCOC_6H_5$-C_o), 128.9 (d, 2-$OCOC_6H_5$-C_m), 133.3 (d, 2-$OCOC_6H_5$-C_p), 169.8 (s, CONH), 12.8 (q, C-1″), 133.0 (s, C-2″), 130.1 (d, C-3″), 13.6 (q, C-4″), 106.3 (d, C-1‴), 79.7 (d, C-2‴), 74.3 (d, C-3‴), 76.9 (d, C-4‴), 65.3 (t, C-5‴), 102.3 (d, C-1⁗), 74.1 (d, C-2⁗), 75.4 (d, C-3⁗), 71.9 (d, C-4⁗), 74.9 (d, C-5⁗), 62.9 (t, C-6⁗); (+)-HR-ESIMS *m/z* 1084.4305 $[M+H]^+$ ($C_{54}H_{70}NO_{22}$, calcd for 1084.4384).

3.3.3. 7-O-[α-D-glucopyranosyl-(1→4)-β-D-xylopyranosyl]-10-deacetyltaxol C (6)

White powder (mp > 200 °C); $[\alpha]^{25}_{D}$ +10 (*c* 0.245, MeOH); IR ν_{max} (micro): 3341, 2927, 1721, 1656, 1270, 1247, 1108, 1056, 709 cm^{-1}; ^1H NMR (600 MHz, pyridine-d_5) δ 6.28 (1H, d, *J* = 6.0 Hz, H-2), 4.42 (1H, m, H-3), 5.26 (1H, br s, H-5), 3.03 (1H, m, H-6a), 2.38 (1H, m, H-6b), 4.76 (1H, dd, *J* = 9.6, 7.2 Hz, H-7), 6.10 (1H, s, H-10), 6.80 (1H, t, *J* = 9.0 Hz, H-13), 2.90 (2H, d, *J* = 8.4 Hz, H-14), 1.64 (3H, s, H-16), 2.33 (3H, s, H-17), 1.55 (3H, s, H-18), 2.21 (3H, s, H-19), 4.58 (1H, dd, *J* = 8.4, 4.8 Hz, H-20a), 4.50 (1H, m, H-20b), 5.26 (1H, s, H-2′), 6.26 (1H, d, *J* = 6.6 Hz, H-3′), 7.35 (2H, d, *J* = 7.8 Hz, 3′-C_6H_5 moiety-H_o), 7.78 (2H, t, *J* = 7.8 Hz, 3′-C_6H_5 moiety-H_m), 7.26 (1H, t, *J* = 7.8 Hz, 3′-C_6H_5 moiety-H_p), 2.54 (3H, s, 4-$OCOCH_3$), 8.35 (2H, d, *J* = 7.8 Hz, 2-$OCOC_6H_5$ moiety-H_o), 7.42 (2H, t, *J* = 7.8 Hz, 2-$OCOC_6H_5$ moiety-H_m), 7.47 (1H, t, *J* = 7.8 Hz, 2-$OCOC_6H_5$ moiety-H_p), 9.17 (1H, d, *J* = 9.6 Hz, NH), 2.38 (2H, m, H-1″), 1.64 (2H, m, H-2″), 1.15 (2H, m, H-3″), 1.11 (2H, m, H-4″), 0.70 (3H, t, *J* = 7.2 Hz, H-3″), 4.57 (1H, d, *J* = 7.8 Hz, 7-xylosyl moiety, H-1‴), 3.96 (1H, m, H-2‴), 3.72 (1H, t, *J* = 7.8 Hz, H-3‴), 3.95 (1H, m, H-4‴), 4.43 (1H, d, *J* = 6.0 Hz, H-5‴a), 3.42 (1H, t, *J* = 10.2 Hz, H-5‴b), 5.61 (1H, d, *J* = 4.2 Hz, Glc-moiety, H-1⁗), 4.08 (1H, dd, *J* = 9.6, 3.6 Hz, H-2⁗), 4.50 (1H, d, *J* = 10.8 Hz, H-3⁗), 4.12 (1H, t, *J* = 8.4 Hz, H-4⁗), 4.34 (1H, m, H-5⁗), 4.50 (1H, m, H-6⁗a), 4.34 (1H, m, H-6⁗b); ^{13}C NMR (150 MHz, pyridine-d_5) δ 78.2 (s, C-1), 76.1 (d, C-2), 47.7 (d, C-3), 81.5 (s, C-4), 84.6 (d, C-5), 36.4 (t, C-6), 82.6 (d, C-7), 57.2 (s, C-8), 210.2 (s, C-9), 75.8 (d, C-10), 137.7 (s, C-11), 138.0 (s, C-12), 72.0 (d, C-13), 36.8 (t, C-14), 44.3 (s, C-15), 21.8 (q, C-16), 14.5 (q, C-17), 27.3 (q, C-18), 11.4 (q, C-19), 76.7 (t, C-20), 174.2 (s, C-1′), 74.6 (d, C-2′), 55.9 (d, C-3′), 140.8 (s, 3′-Ph-C_q), 128.8 (d, 3′-Ph-C_o), 128.0 (d, 3′-Ph-C_m), 127.7 (d, 3′-Ph-C_p), 173.2 (s, 4-O$\underline{C}OCH_3$), 22.8 (q, 4-OCO$\underline{C}H_3$), 166.4 (s, 2-O$\underline{C}OC_6H_5$), 131.2 (s, 2-OCO$\underline{C_6H_5}$-C_q), 130.5 (d, 2-OCO$\underline{C_6H_5}$-C_o), 128.9 (d, 2-OCO$\underline{C_6H_5}$-C_m), 133.3 (d, 2-OCO$\underline{C_6H_5}$-C_p), 170.7 (s, $\underline{C}$ONH), 36.5 (t, C-1″), 26.0 (t, C-2″), 31.6 (t, C-3″), 22.6 (t, C-4″), 14.0 (q, C-5″), 106.5 (d, C-1‴), 79.7 (d, C-2‴), 74.3 (d, C-3‴), 76.9 (d, C-4‴), 65.3 (t, C-5‴), 102.3 (d, C-1⁗), 74.1 (d, C-2⁗), 75.4 (d, C-3⁗), 71.9 (d, C-4⁗), 74.9 (d, C-5⁗), 62.9 (t, C-6⁗); (+)-HR-ESIMS *m/z* 1100.4618 $[M+H]^+$ ($C_{55}H_{74}NO_{22}$, calcd for 1100.4697).

3.4. Acid hydrolysis and sugar analysis

Compounds **4**–**6** (ca. 1 mg) were individually hydrolyzed with 2 M TFA (3 mL) at 120 °C for 3 h. The reaction mixture was extracted with EtOAc, and the H_2O layer was concentrated under vacuo to give residue. The residue was dissolved with 0.5 mL pyridine. To the solution, were added 10 mg of hydroxylamine hydrochloric acid and 0.5 mL of AcO_2, and the mixture was stirred at 90 °C for 30 min. The reaction solution was concentrated, dissolved with $CHCl_3$ and subjected to GC-MS analysis. The same procedure was applied to the authentic samples (D-glucose and D-xylose).

3.5. Cytotoxicity bioassays of metabolites

The HCT-8 human colorectal adenocarcinoma cell line, the Bel-7402 human liver cancer cell line, and the BGC-823 human gastric cancer cell line were purchased from the Institute of Cell Biology (Shanghai, China); the A549 human lung carcinoma cell line, and the A2780 human ovarian cancer cell line were obtained from ATCC. All five tumor cell lines were maintained in RRMI1640 medium supplemented with 10% (v/v) fetal bovine serum (FBS), 100 units/mL penicillin, and 100 μg/mL streptomycin. Cultures were incubated at 37 °C in a humidified 5% CO_2 atmosphere. The tumor cells were seeded in 96-well microtiter plates at 1200 cells/well. After 24 h, the compounds were added to the cells. After incubation for 96 h, cell viability was determined by measuring the metabolic conversion of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) into purple formazan crystals by active cells. The MTT assay results were read using an MK 3 wellscan (Labsystem Drogon) plate reader at 570 nm. All compounds were tested at five concentrations (10^{-5}, 10^{-6}, 10^{-7}, 10^{-8}, 10^{-9} M) and were dissolved in 100% DMSO with a final concentration of DMSO of 0.1% (v/v) in each well. Each concentration of the compounds was tested in three parallel wells. IC_{50} values were calculated using Microsoft Excel software.

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Appendix A. Supplementary data

The HPLC chromatogram of biotransformation of **1**, **2**, and **3** by *Enterobacter cloaceae* CGMCC 1.181; the copies of ^1H and ^{13}C NMR spectra of new compounds **4**–**6**; GC-MS spectra of the sugar derivatives obtained from acid hydrolysis of **4**. Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.fitote.2012.11.006.

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