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Original article

Synthesis and antitumor activity of new shikonin glycosides

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ABSTRACT

Eleven shikonin glycosides were synthesized and evaluated for their antitumor activity in vitro. Some of them were found to exhibit cytotoxic activities against both drug sensitive cell lines (K562, MCF-7 and HL60) and their drug resistant cell sublines (K562/ADR, MCF-7/ADR and HL60/ADR).

1. Introduction

Shikonin, one of the active components isolated from the traditional medicinal herb *Lithospermium erythrorhizon* [1], has attracted much attention due to its significant biological activities such as anti-inflammatory [2], antibacterial [3], wound healing [4] and immunostimulatory activities [5]. It was also reported that shikonin exhibited anticancer activity by inhibiting telomerase and DNA topoisomerase I/II [6], and cancer cell growth [7]. However, its poor toxicity and solubility prevent shikonin from being an anticancer drug. Many efforts have been made to synthesize new derivatives of shikonin, aiming at discovering more effective antitumor drugs [8–10]. Previously, we reported that shikonin could circumvent cancer drug resistance by induction of a necroptotic death [11]. Encouraged by this result, we are interested in the synthesis and evaluation of new shikonin derivatives. We synthesized eleven shikonin glycosly derivatives possessing acylglycosly at 1′-OH and tested their in vitro cytotoxicity on three pairs of drug sensitive and drug resistant cell lines. These results showed all of the shikonin acylglycosyl derivatives exhibited similar or stronger cytotoxicity than shikonin and provided a good lead for the search of more effective antitumor drug.

2. Chemistry

Shikonin was glycosylated at 1′-OH to maintain the quinine moiety, which has been identified as a pharmacophore that commonly affords cytotoxicity [12]. Synthesis of acetyl-β-glycosyl shikonins is depicted in Scheme 1.

D-Glucopyranose (**1a**), D-galactopyranose (**1b**), D-mannopyranose (**1c**), L-rhampyranose (**1d**), D-xylopyranose (**1e**), D-ribofuranose (**1f**), D-arabofuranose (**1g**), L-arabofuranose (**1h**), cellubiose (**1i**), maltose (**1j**), and lactose (**1k**) were acetylated with acetic anhydride and anhydrous sodium acetate to give the corresponding acetylated sugars **2a–k**. The anomeric *O*-acetyl groups of **2a–e** and **2i–k** were selectively removed with hydrazine acetate to afford **3a–e** and **3i–k** [13], while the anomeric *O*-acetyl groups of furanoses **2f–h** were removed with $NaOCH_3$ in THF at 0 °C to afford **3f–h** [14]. Treatment **3** with trichloroacetonitrile and potassium carbonate yielded acetyl-α-glycosyl tirchloroacetimates **4** [15]. Compounds **4** as glycosyl donors reacted with shikonin under $BF_3 \cdot EtO_2$ and 4 Å molecular sieve at nitrogen atmosphere to furnish the corresponding shikonin acetyl-β-glycosides **5a–k** according to the published method [16]. The glycosylation of shikonin was highly regioselective. The reaction occurred almost at the side chain hydroxyl (1′-OH) of shikonin, due to the hydrogen bond between phenolic hydroxyl and quinoid carbonyl reducing the nucleophilicity of phenolic hydrogen.

All new products were well characterized by ^1H NMR, ^{13}C NMR and IR spectroscopy as well as high-resolution mass spectrometry.

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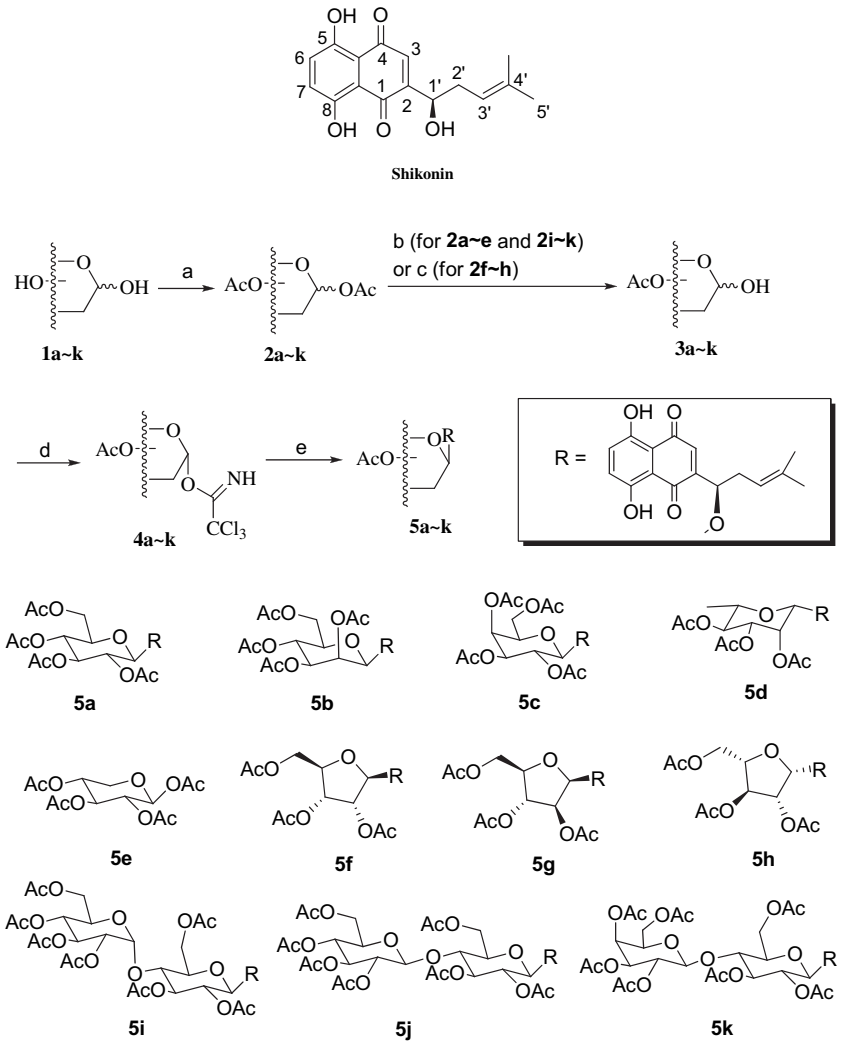

Scheme 1. Synthesis of acetyl-β-glycosyl shikonins **5a–k**. Reagents and conditions: (a) Ac_2O/AcONa, 80 °C, 4 h, 90–98%; (b) AcOH.NH_2NH_2, THF, r.t., 4 h, 75%–90%; (c) $NaOCH_3$, THF, 0 °C, 30 min, 67–81%; (d) Cl_3CCN, K_2CO_3, DCM, r.t., overnight, 60–85%; (e) $BF_3{\cdot}EtO_2$, shikonin, 4 Å MS, DCM, −40 °C, 1 h, 46–87%.

3. Results and discussions

In comparison to shikonin, the derivatives have similar or stronger inhibitory activities against both drug sensitive cell lines (K562, MCF-7 and HL60) and their drug resistant cell sublines: K562/ADR, MCF-7/ADR and HL60/ADR (Table 1).

Cancer drug resistance is a major problem limiting cancer chemotherapeutic efficacy [17]. Overexpression of drug transporters such as P-gp and MRP1, is one of the common mechanisms of cancer multidrug resistance. P-gp and MRP1 are transmembrane proteins and function as unilateral drug transporters that expel intracellular drugs out of cells, so that intracellular drug concentration may be kept at sublethal concentration. Not less importantly, these drug transporters have broad substrate specificity and thus can recognize many structurally and functionally unrelated anticancer agents, leading to multidrug

Table 1
In vitro cytotoxicity of shikonin derivatives.

Compounds	IC_{50}(μM)					
	K562	K562/ADR (drug resistance factor)[a]	MCF-7	MCF-7/Adr (drug resistance factor)[a]	HL60	HL60/Adr (drug resistance factor)[a]
SH	0.63	0.67 (1.06)	1.45	1.65 (1.14)	1.03	1.41 (1.37)
5a	0.30	0.73 (2.38)	0.63	0.47 (0.75)	0.35	0.34 (0.99)
5b	0.35	0.75 (2.12)	0.58	0.71 (1.24)	0.37	0.44 (1.18)
5c	0.43	0.80 (1.86)	0.51	0.95 (1.86)	1.17	1.36 (1.16)
5d	0.26	0.64 (2.46)	0.40	0.60 (1.50)	0.33	0.36 (1.09)
5e	0.20	0.38 (1.63)	0.38	0.90 (2.40)	0.33	0.36 (1.11)
5f	0.23	0.54 (2.35)	0.30	0.55 (1.83)	0.62	0.56 (0.90)
5g	0.25	0.75 (3.00)	0.38	0.59 (1.55)	0.79	1.38 (1.75)
5h	0.44	0.69 (1.57)	0.46	1.14 (2.48)	1.04	1.13 (1.09)
5i	0.31	0.98 (3.14)	0.44	1.81 (4.16)	0.28	0.33 (1.16)
5j	0.22	1.22 (5.54)	0.23	0.26 (1.13)	0.70	1.10 (1.57)
5k	0.18	0.64 (3.53)	0.28	1.17 (4.18)	0.16	0.25 (1.52)

[a] Drug resistance factor = $IC_{50\ \text{resistant cell line}}/IC_{50\ \text{sensitive cell line}}$.

resistance. K562/ADR and MCF-7/ADR are cell lines with characteristics of P-gp overexpression, and HL60/ADR with MRP1 overexpression. Since shikonin derivatives have similar cytotoxic against both drug sensitive and resistant cells, i.e., overexpression of P-gp and MRP1 does not affect their cellular cytotoxicity, these compounds have potential to treat drug resistant cancer.

4. Conclusion

In summary, a novel series of shikonin glycosides have been synthesized and assessed for their preliminary anticancer activity against both drug sensitive cell lines (K562, MCF-7 and HL60) and their drug resistant cell sublines (K562/ADR, MCF-7/ADR and HL60/ADR). Most of the shikonin derivatives exhibited good cytotoxicity against all three cancer cell lines evaluated. The results suggest that some of the synthesized glycosides, such as **5e** and **5k**, should be potential compounds for treatment of certain drug resistant cancers.

5. Experimental

5.1. Chemistry

All reactions were performed under nitrogen atmosphere. The reactions were monitored by TLC on silica gel GF254. Detection was effected by examination under UV light and by sprayed with 20% concd. H_2SO_4 in EtOH and heated at 110 °C. Column chromatography was performed on silica gel H. Intermediates and products synthesized were characterized based on ^{1}H NMR (bruker AM400) and ^{13}C NMR. $CDCl_3$ was used as common solvent for the NMR. Infrared (IR) spectra (KBr) were recorded on a Nicolet-470 Spectrometer. Melting points were determined by micro melting point apparatus. Mass spectra (MS) were recorded on a Bruker Esruire 3000 Plus mass spectrometer through electron spray ionization. High resolution mass spectral (HRMS) analyses were recorded on BRUKER DALTONICS APEX III mass spectrometer using electron spray ionization.

5.2. General method for the synthesis of compounds **2a–k**

To glucose (10 mmol) and anhydrous sodium acetate (13 mmol) was added acetic anhydride (100 mmol). The mixture was stirred at 80 °C for 4 h (reaction progress was monitored by TLC). After cooled to room temperature, the reaction solution was pounded into cold saturated sodium bicarbonate solution. The mixture was stirred for 1.5 h, extracted with dichloromethane and dried over sodium sulfate. The solvent was removed in vacuum and the residue was purified by flash silica gel column chromatography (AcOEt/petroleum ether = 1:4, v/v).

5.2.1. 1,2,3,4,6-Penta-O-acetylglucopyranose (**2a**)

Yield 93%, white solid, mp: 93–95 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.72 (d, J = 8.0 Hz, 1H), 5.26 (t, J = 9.6 Hz, 1H), 5.14 (m, 2H), 4.30 (dd, J = 4.4, 12.4 Hz, 1H), 4.11 (dd, J = 1.6, 12.4 Hz, 1H), 3.85 (m, 1H), 2.12 (s, 3H), 2.09 (s, 3H), 2.04 (s, 6H), 2.02 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.6, 170.0, 169.3, 169.2, 168.9, 91.6, 72.7, 72.6, 70.1, 67.6, 61.3, 20.8, 20.7, 20.5.

5.2.2. 1,2,3,4,6-Penta-O-acetylmannopyranose (**2b**)

Yield 98%, white solid, mp: 111–113 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.96 (m, 1H), 5.22 (d, J = 6.4 Hz, 2H), 5.13 (s, 1H), 4.16 (dd, J = 4.8, 12.4 Hz, 1H), 3.97 (m, 2H), 2.06 (s, 3H), 2.05 (s, 3H), 1.97 (s, 6H), 1.94 (s, 3H), 1.89 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.2, 170.0, 169.4, 169.2, 168.8, 92.0, 71.5, 70.6, 67.6, 66.6, 60.9, 20.7, 20.5, 20.5, 20.4.

5.2.3. 1,2,3,4,6-Penta-O-acetylgalactopyranose (**2c**)

Yield 96%, white solid, mp: 134–136 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.48 (d, J = 8.4 Hz, 1H), 5.20 (s, 1H), 5.11 (t, J = 9.6 Hz, 1H), 4.85 (dd, J = 2.4, 10.0 Hz, 1H), 3.90 (m, 3H), 1.94 (s, 3H), 1.90 (s, 3H), 1.82 (s, 6H), 1.77 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.2, 169.6, 169.4, 169.2, 167.7, 92.3, 70.2, 68.4, 68.0, 65.1, 61.7, 20.5, 20.4, 20.4, 20.3, 20.2.

5.2.4. 1,2,3,4-Tetra-O-acetylrhampyranose (**2d**)

Yield 98%, Colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.81 (s, 1H), 5.44 (s, 1H), 5.05 (m, 2H), 3.65 (m, 1H), 2.18 (s, 3H), 2.08 (s, 3H), 2.03 (s, 3H), 1.98 (s, 3H), 1.26 (d, J = 6.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.2, 169.8, 168.7, 168.4, 90.2, 71.4, 70.6, 70.2, 68.4, 20.7, 20.7, 20.6, 20.4, 17.3.

5.2.5. 1,2,3,4-Tetra-O-acetylxylopyranose (**2e**)

Yield 95%, white solid, mp: 122–124 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.70 (d, J = 6.8 Hz, 1H), 5.19 (t, J = 8.0 Hz, 1H), 5.00 (m, 2H), 4.13 (dd, J = 4.8, 12.0 Hz, 1H), 3.51 (dd, J = 8.0, 12.0 Hz, 1H), 2.09 (s, 3H), 2.04 (s, 3H), 2.04 (s, 3H), 2.03 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.6, 169.1, 168.8, 91.9, 70.9, 69.4, 68.2, 62.6, 20.6, 20.5, 20.5, 20.4.

5.2.6. 1,3,4-Tetra-O-acetyl-D-ribofuranose (**2f**)

Yield 97%, white solid, mp: 109–111 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.00 (d, J = 4.0 Hz, 1H), 5.46 (d, J = 2.8 Hz, 1H), 5.13 (m, 1H), 5.02 (d, J = 2.8 Hz, 1H), 4.01 (m, 1H), 3.89 (m, 1H), 2.11 (s, 3H), 2.08 (m, 9H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.8, 169.7, 169.4, 168.7, 90.8, 67.2, 66.0, 62.6, 20.8, 20.7, 20.6, 20.5.

5.2.7. 1,2,3,4-Tetra-O-acetyl-D-arabofuranose (**2g**)

Yield 90%, Colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.68 (d, J = 6.4 Hz, 1H), 5.32 (m, 2H), 5.13 (dd, J = 3.6 8.8 Hz, 1H), 4.06 (dd, J = 3.6 13.2 Hz, 1H), 3.79 (dd, J = 1.2 13.2 Hz, 1H), 2.16 (s, 3H), 2.14 (s, 3H), 2.08 (s, 3H), 2.06 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.1, 169.8, 169.3, 169.0, 92.0, 69.8, 67.9, 67.0, 63.8, 20.8, 20.7, 20.6, 20.5.

5.2.8. 1,2,3,4-Tetra-O-acetyl-L-arabofuranose (**2h**)

Yield 91%, white solid, mp: 89–90 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.64 (d, J = 6.8 Hz, 1H), 5.25 (m, 2H), 5.08 (dd, J = 3.2 8.8 Hz, 1H), 4.01 (dd, J = 3.6 12.8 Hz, 1H), 3.74 (d, J = 13.2 Hz, 1H), 2.11 (s, 3H), 2.09 (s, 3H), 2.04 (s, 3H), 2.01 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.1, 169.8, 169.3, 169.0, 92.1, 69.8, 68.0, 67.1, 63.8, 20.8, 20.7, 20.6, 20.5.

5.2.9. 1,2,3,6,2′,3′,4′,6′-Octa-O-acetylmaltose (**2i**)

Yield 98%, white solid, mp: 163–165 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.73 (d, J = 7.6 Hz, 1H), 5.40 (d, J = 3.6 Hz, 1H), 5.34 (t, J = 5.6 Hz, 1H), 5.29 (t, J = 8.8 Hz, 1H), 5.05 (t, J = 10.0 Hz, 1H), 4.97 (t, J = 8.8 Hz, 1H), 4.85 (dd, J = 4.0, 10.4 Hz, 1H), 4.44 (d, J = 10.6 Hz, 1H), 4.22 (m, 2H), 4.04 (m, 2H), 3.92 (d, J = 10.8 Hz, 1 H), 3.83 (d, J = 9.6 Hz, 1H), 2.13 (s, 3H), 2.09 (s, 6H), 2.04 (s, 3H), 2.02 (s, 3H), 2.01 (s, 6H), 2.00 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.5, 170.4, 170.3, 170.0, 169.8, 169.5, 169.3, 168.7, 95.6, 91.1, 75.1, 72.8, 72.2, 70.8, 69.9, 69.2, 68.4, 67.8, 62.4, 61.3, 20.8, 20.7, 20.6, 20.5, 20.4.

5.2.10. 1,2,3,6,2′,3′,4′,6′-Octa-O-acetylcellubiose (**2j**)

Yield 96%, white solid, mp: 188–190 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.64 (d, J = 8.0 Hz, 1H), 5.21 (t, J = 5.2 Hz, 1H), 5.04 (m, 3H), 4.90 (t, J = 8.0 Hz, 1H), 4.48 (m, 2H), 4.34 (dd, J = 4.4, 12.0 Hz, 1H), 4.10 (dd, J = 4.4, 12.0 Hz, 1H), 4.03 (d, J = 11.2 Hz, 1H), 3.80 (t, J = 9.6 Hz, 1H), 3.66 (m, 2H), 2.16 (s, 3H), 2.11 (s, 3H), 2.10 (s, 3H), 2.01 (m, 9H), 1.99 (s, 3H), 1.96 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.3, 170.1, 170.0, 169.5, 169.3, 169.1, 168.8, 168.7, 100.5, 91.4, 75.7, 73.4, 72.7, 72.2, 71.8, 71.4, 70.3, 67.7, 61.4, 20.7, 20.6, 20.5, 20.4, 20.3.

*5.2.11. 1,2,3,6,2′,3′,4′,6′-Octa-O-acetyllactose (**2k**)*

Yield 93%, white solid, mp: 130–132 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.67 (d, J = 8.4 Hz, 1H), 5.35 (d, J = 3.2 Hz, 1H), 5.25 (d, J = 9.2 Hz, 1H), 5.05 (m, 3H), 4.47 (m, 2H), 4.12 (m, 3H), 3.85 (m, 3H), 2.16 (s, 3H), 2.12 (s, 3H), 2.10 (s, 3H), 2.07 (s, 3H), 2.06 (s, 3H), 2.05 (s, 3H), 2.04 (s, 3H), 1.97 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.3, 170.2, 170.1, 170.0, 169.6, 169.5, 168.9, 168.8, 100.9, 91.4, 75.6, 73.4, 72.5, 70.8, 70.6, 70.4, 68.8, 66.5, 61.6, 60.7, 20.9, 20.8, 20.7, 20.6, 20.6, 20.5, 20.4.

*5.3. General method for the synthesis of compounds **4a–e** and **4i–k***

A solution of **2** (5 mmol) and hydrazine acetate (5.5 mmol) in THF (20 mL) was stirred for 4 h at room temperature. After removed the solvent, dichloromethane (40 mL) was added to dissolve the residue. The organic phase was washed with water (10 mL), dried over anhydrous sodium sulfate. The solvent was removed in vacuum to give **3**. K_2CO_3 (15 mmol) and trichloroacetonitrile (12 mmol) was added to the solution of **3** (3 mmol) in anhydrate dichloromethane (40 mL). The mixture was stirred at room temperature overnight. The resulting mixture was filtered to give the organic phase. The organic phase was concentrated in vacuum and the residue was purified by flash silica gel column chromatography (AcOEt/petroleum ether = 1:4, v/v).

*5.3.1. 2,3,4,6-Tetra-O-acetyl-α-glucopyranosyl trichloroacetimidate (**4a**)*

Yield 70%, white solid, mp: 66–68 °C; ^{1}H NMR (400 MHz, CDCl3): δ 8.69 (s, 1H), 6.56 (d, J = 3.6 Hz, 1H), 5.57 (t, J = 9.6 Hz, 1H), 5.19 (t, J = 9.6 Hz, 1H), 5.13 (dd, J = 3.6, 10.0 Hz, 1H), 4.28 (dd, J = 4.0, 12.4 Hz, 1H), 4.22 (m, 1H), 4.12 (dd, J = 2.0, 12.4 Hz, 1H), 2.08 (s, 3H), 2.05 (s, 3H), 2.03 (s, 3H), 2.02 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.5, 170.0, 169.8, 169.4, 160.7, 92.8, 90.6, 69.9, 69.8, 69.6, 67.7, 61.3, 20.6, 20.5, 20.4.

*5.3.2. 2,3,4,6-Tetra-O-acetyl-α-mannopyranosyl trichloroacetimidate (**4b**)*

Yield 62%, white solid, mp: 62–64 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.78 (s, 1H), 6.27 (s, 1H), 5.46 (s, 1H), 5.39 (m, 2H), 4.26 (m, 1H), 4.16 (m, 2H), 2.19 (s, 3H), 2.07 (s, 3H), 2.05 (s, 3H), 2.00 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.5, 169.8, 169.6, 169.5, 159.6, 94.4, 90.4, 71.1, 68.7, 67.7, 65.2, 61.9, 20.7, 20.6 20.5.

*5.3.3. 2,3,4,6-Tetra-O-acetyl-α-galactopyranosyl trichloroacetimidate (**4c**)*

Yield 68%, white solid, mp: 112–114 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.66 (s, 1H), 6.59 (d, J = 3.2 Hz, 1H), 5.55 (d, J = 2.8 Hz, 1H), 5.37 (m, 2H), 4.43 (t, J = 6.8 Hz, 1H), 4.15 (dd, J = 2.4, 11.2 Hz, 1H), 4.07 (dd, J = 6.8, 11.2 Hz, 1H), 2.16 (s, 3H), 2.02 (s, 3H), 2.01 (s, 3H), 2.00 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.2, 170.0, 170.0, 169.9, 160.8, 93.4, 90.6, 68.9, 67.4, 67.2, 66.8, 61.2, 20.6, 20.5, 20.5, 20.4.

*5.3.4. 2,3,4-Tri-O-acetyl-α-rhampyranosyl trichloroacet-imidate (**4d**)*

Yield 66%, white solid, mp: 78–80 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.74 (s, 1H), 6.21 (s, 1H), 5.47 (d, J = 2.0 Hz, 1H), 5.37 (dd, J = 10.4, 4.0 Hz, 1H), 5.18 (d, J = 10.4 Hz, 1H), 4.10 (m, 1H), 2.19 (s, 3H), 2.08 (s, 3H), 2.01 (s, 3H), 1.28 (d, J = 6 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.9, 169.8, 169.7, 159.9, 94.6, 90.6, 70.2, 69.2, 68.8, 68.1, 20.8, 20.7 20.6, 17.4.

*5.3.5. 2,3,4-Tri-O-acetyl-α-xylopyranosyl trichloroacet-imidate (**4e**)*

Yield 59%, white solid, mp: 96–97 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.65 (s, 1H), 6.47 (d, J = 3.2 Hz, 1H), 5.56 (t, J = 5.6 Hz, 1H), 5.07 (m, 2H), 3.98 (dd, J = 6.4, 10.8 Hz, 1H), 3.80 (t, J = 6.8 Hz, 1H), 2.05 (s, 3H), 2.04 (s, 3H), 2.00 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.8, 169.7, 160.9, 93.1, 90.7, 69.8, 69.3, 68.5, 60.7, 20.6, 20.5 20.4.

*5.3.6. 2,3,6,2′,3′,4′,6′-Hepta-O-acetyl-α-maltosyl trichloroacetimidate (**4i**)*

Yield 66%, white solid, mp: 79–81 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ8.65 (s, 1H), 6.45 (d, J = 4.0 Hz, 1H), 5.58 (t, J = 9.6 Hz, 1H), 5.42 (d, J = 4.4 Hz, 1H), 5.36 (t, J = 10.0 Hz, 1H), 5.04 (t, J = 9.6 Hz, 1H), 4.98 (dd, J = 3.6, 10.0 Hz, 1H), 4.85 (dd, J = 3.6, 10.8 Hz, 1H), 4.46 (d, J = 12.0 Hz, 1H), 4.21 (m, 3H), 4.04 (m, 2H), 3.92 (d, J = 10.0 Hz, 1H), 2.10 (s, 3H), 2.07 (s, 3H), 2.04 (s, 3H), 2.01 (s, 3H), 2.00 (s, 3H), 1.98 (s, 3H), 1.96 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.5, 170.4, 170.2, 169.8, 169.5, 169.3, 160.7, 95.6, 92.6, 90.5, 72.0, 71.8, 70.4, 70.0, 69.8, 69.2, 68.4, 67.8, 62.1, 61.2, 20.8, 20.6, 20.5, 20.4, 20.3, 20.2.

*5.3.7. 2,3,6,2′,3′,4′,6′-Hepta-O-acetyl-α-cellubiosyl trichloroacetimidate (**4j**)*

Yield 60%, white solid, mp: 111–113 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ8.66 (s, 1H), 6.48 (d, J = 2.8 Hz, 1H), 5.53 (t, J = 6.4 Hz, 1H), 5.08 (m, 3H), 4.93 (t, J = 8.4 Hz, 1H), 4.54 (t, J = 8.4 Hz, 2H), 4.39 (dd, J = 4.0, 12.0 Hz, 1H), 4.11 (m, 3H), 3.84 (t, J = 10.0 Hz, 1H), 3.67 (d, J = 9.2 Hz, 1H), 2.11 (s, 3H), 2.09 (s, 3H), 2.04 (s, 3H), 2.03 (s, 3H), 2.01 (s, 6H), 1.98 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.4, 170.2, 170.1, 170.0, 169.4, 169.2, 169.0, 160.9, 100.9, 92.8, 76.0, 73.0, 72.0, 71.0, 71.6, 70.9, 69.8, 69.2, 61.5, 61.3, 20.7, 20.6, 20.5, 20.4, 20.3.

*5.3.8. 2,3,6,2′,3′,4′,6′-Hepta-O-acetyl-α-lactosyl trichloroacetimidate (**4k**)*

Yield 69%, white solid, mp: 153–155 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.66 (s, 1H), 6.46 (d, J = 3.6 Hz, 1H), 5.59 (t, J = 9.6 Hz, 1H), 5.42 (d, J = 4.4 Hz, 1H), 5.37 (t, J = 9.6 Hz, 1H), 5.06 (t, J = 9.6 Hz, 1H), 5.01 (dd, J = 3.6, 9.6 Hz, 1H), 4.86 (dd, J = 4.0, 10.4 Hz, 1H), 4.48 (d, J = 13.2 Hz, 1H), 4.23 (m, 3H), 4.05 (t, J = 10.0 Hz, 2H), 3.93 (d, J = 9.6 Hz, 1H), 2.11 (s, 3H), 2.08 (s, 3H), 2.05 (s, 3H), 2.02 (s, 3H), 2.00 (s, 3H), 1.99 (s, 3H), 1.97 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.5, 170.4, 170.3, 169.9, 169.6, 169.4, 160.8, 95.7, 92.7, 90.6, 72.1, 71.9, 70.5, 70.1, 69.9, 69.3, 68.5, 67.9, 62.2, 61.3, 20.8, 20.7, 20.6, 20.5, 20.3.

*5.4. General method for the synthesis of compounds **4f–h***

A mixture of **2f–h** (5 mmol) and NaOMe (10 mmol) in THF (20 mL) was stirred at 0 °C for 30 min and then neutralized with AcOH (10 mmol). After removed the solvent in vacuum, dichloromethane (40 ml) was added to dissolve the residue. The organic phase was washed with water (10 mL) and dried over anhydrous sodium sulfate. The solvent was removed in vacuum to give **3f–h**. K_2CO_3 (15 mmol) and trichloroacetonitrile (12 mmol) was added to the solution of **3f–h** (3 mmol) in anhydrate dichloromethane (20 mL). The mixture was stirred at room temperature overnight. The resulting mixture was filtered to give the organic phase. After removed the solvent in vacuum, the residue was purified by flash silica gel column chromatography (AcOEt/petroleum ether = 1:4, v/v).

*5.4.1. 2,3,5-Tri-O-acetyl-α-D-ribofuranosyl trichloro-acetimidate (**4f**)*

Yield 49%, Colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.74 (s, 1H), 6.22 (d, J = 3.6 Hz, 1H), 5.46 (t, J = 3.6 Hz, 1H), 5.26 (t, J = 3.6 Hz, 1H), 5.22 (d, J = 3.6 Hz, 1H), 5.17 (dd, J = 2.8 12.8 Hz, 1H), 3.97 (dd, J = 4.4 12.8 Hz, 1H), 2.12 (s, 3H), 2.11 (s, 3H), 2.05 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.9, 169.6, 169.5, 160.2, 95.1, 90.5, 66.6, 66.2, 65.6, 63.2, 20.8, 20.6, 20.5.

*5.4.2. 2,3,5-Tri-O-acetyl-α-D-arabofuranosyl trichloro-acetimidate (**4g**)*

Yield 46%, white solid, mp: 130–132 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.61 (s, 1H), 6.52 (d, J = 2.8 Hz, 1H), 5.38 (m, 3H), 4.12

(d, $J = 13.2$ Hz, 1H), 3.84 (d, $J = 13.2$ Hz, 1H), 2.13 (s, 3H), 2.00 (s, 3H), 1.99 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.1, 170.0, 169.8, 160.9, 94.0, 90.8, 68.2, 67.1, 67.0, 62.7, 20.8, 20.6, 20.4.

*5.4.3. 2,3,5-Tri-O-acetyl-α-L-arabofuranosyl trichloro-acetimidate (**4h**)*

Yield 57%, white solid, mp: 106–107 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.72 (s, 1H), 5.95 (d, $J = 4.4$ Hz, 1H), 5.31 (m, 2H), 5.22 (dd, $J = 3.2$ 6.4 Hz, 1H), 4.14 (dd, $J = 7.2$ 12.0 Hz, 1H), 3.80 (dd, $J = 3.2$ 12.0 Hz, 1H), 2.16 (m, 6H), 2.01 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.8, 169.7, 168.9, 160.5, 94.4, 90.6, 68.0, 67.8, 65.5, 61.0, 20.7, 20.6, 20.5.

*5.5. General method for the synthesis of compounds **5a–k***

To the mixture of **4** (1 mmol), shikonin (1 mmol) and 4 Å molecular sieve (0.4 g) in dichloromethane (5 mL) was added dropwise a solution of $BF_3 \cdot Et_2O$ (0.1 mmol) in dichloromethane (3 mL) at −40 °C. After another hour of stirring, Et_3N (1 mmol) was added to the mixture, and AcOH (1 mmol) was added. The reaction mixture was filtered to give the organic phase. Removed the solvent of organic phase in vacuum and the residue was purified by flash chromatography to afford **5a–k** (51–87%).

*5.5.1. 1-(1-(5,8-Dihydroxy-1,4-naphthoquinone-2-yl)-4-methyl-3-pentenyloxy)-2,3,4,6-tetra-O-acetyl-β-glucopyranoside (**5a**)*

Yield 86%, red solid, mp: 58–60 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ12.49(s, 1H), 12.41 (s, 1H), 7.12 (m, 3H), 5.13 (m, 1H), 5.00 (m, 3H), 4.86 (m, 1H), 4.60 (d, $J = 8.0$ Hz, 1H), 3.95 (m, 2H), 3.53 (m, 1H), 2.45 (m, 1H), 2.26 (m, 1H), 2.00 (s, 3H), 1.95 (s, 3H), 1.94 (s, 3H), 1.85 (s, 3H), 1.62 (s, 3H), 1.49 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 180.4, 179.2, 170.3, 170.2, 169.3, 169.1, 165.3, 164.8, 149.7, 135.2, 133.6, 132.3, 131.7, 118.1, 111.8, 111.5, 100.8, 75.6, 72.6, 71.8, 71.4, 68.2, 61.5, 33.7, 25.7, 20.6, 20.5, 20.4, 20.3, 17.9; HRMS (ESI) Calcd for $[M + Na]^+ = 641.1841$, found: $[M + Na]^+ = 641.1864$; IR (KBr) ν (cm^{-1}) = 3467, 1750, 1611, 1455, 1229, 1042.

*5.5.2. 1-(1-(5,8-Dihydroxy-1,4-naphthoquinone-2-yl)-4-methyl-3-pentenyloxy)-2,3,4,6-tetra-O-acetyl-β-manno-pyranoside (**5b**)*

Yield 46%, red solid, mp: 113–115 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 12.57 (s, 1H), 12.46 (s, 1H), 7.18 (m, 3H), 5.30 (m, 2H), 5.23 (t, $J = 10$ Hz, 1H), 5.12 (t, $J = 6.8$ Hz, 1H), 5.04 (m, 2H), 4.14 (dd, $J = 5.6$, 12.0 Hz, 1H), 3.89 (dd, $J = 1.6$, 12.4 Hz, 1H), 3.83 (m, 1H), 2.51 (m, 1H), 2.45 (m, 1H), 2.14 (s, 3H), 2.02 (s, 3H), 2.00 (s, 3H), 1.94 (s, 3H), 1.67 (s, 3H), 1.58 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 177.9, 176.4, 170.4, 169.9, 169.7, 169.7, 167.8, 167.3, 148.9, 136.6, 133.1, 132.7, 132.1, 117.4, 111.7, 111.5, 97.5, 73.2, 69.4, 69.3, 68.9, 66.0, 62.4, 33.5, 25.7, 20.8, 20.7, 20.6, 20.4, 18.0; HRMS (ESI) Calcd for $[M + Na]^+ = 641.1841$, found: $[M + Na]^+ = 641.1853$; IR (KBr) ν (cm^{-1}) = 3483, 1758, 1611, 1451, 1215, 1053.

*5.5.3. 1-(1-(5,8-Dihydroxy-1,4-naphthoquinone-2-yl)-4-methyl-3-pentenyloxy)-2,3,4,6-tetra-O-acetyl-β-galacto-pyranoside (**5c**)*

Yield 87%, red solid, mp: 61–63 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 12.58 (s, 1H), 12.51 (s, 1H), 7.21 (m, 3H), 5.36 (m, 1H), 5.27 (dd, $J = 10.4$, 8.0 Hz, 1H), 5.14 (t, $J = 6.8$ Hz, 1H), 5.02 (dd, $J = 3.2$, 6.4 Hz, 1H), 4.97 (dd, $J = 4.0$, 6.8 Hz, 1H), 4.62 (d, $J = 7.6$ Hz, 1H), 3.99 (d, $J = 6.4$ Hz, 2H), 3.83 (t, $J = 6.4$ Hz, 1H), 2.52 (m, 1H), 2.35 (m, 1H), 2.17 (s, 3H), 2.09 (s, 3H), 2.00 (s, 3H), 1.92 (s, 3H), 1.69 (s, 3H), 1.56 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 180.4, 179.2, 170.2, 170.1, 170.0, 169.1, 165.1, 164.6, 149.5, 135.1, 133.6, 132.1, 131.6, 117.9, 111.6, 111.4, 101.1, 75.3, 70.7, 70.6, 68.7, 66.7, 61.0, 33.4, 25.7, 20.6, 20.5, 20.4, 20.3, 17.8; HRMS (ESI) Calcd for $[M + Na]^+ = 641.1841$, found: $[M + Na]^+ = 641.1856$; IR (KBr) ν (cm^{-1}) = 3468, 1753, 1612, 1455, 1221, 1079.

*5.5.4. 1-(1-(5,8-Dihydroxy-1,4-naphthoquinone-2-yl)-4-methyl-3-pentenyloxy)-2,3,4-tri-O-acetyl-β-rhampyranoside (**5d**)*

Yield 82%, red solid, mp: 117–118 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 12.60 (s, 1H), 12.48 (s, 1H), 7.19 (s, 2H), 7.17 (s, 1H), 5.26 (m, 2H), 5.11 (t, $J = 3.2$ Hz, 1H), 5.03 (m, 2H), 4.93 (s, 1H), 3.73 (m, 1H), 2.47 (m, 2H), 2.15 (s, 3H), 2.04 (s, 3H), 2.00 (s, 3H), 1.67 (s, 3H), 1.58 (s, 3H), 1.03 (d, $J = 3.2$ Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 178.4, 176.9, 169.8, 169.8, 169.7, 167.1, 166.6, 149.1, 136.2, 132.7, 132.4, 132.1, 117.4, 111.6, 111.4, 97.4, 73.1, 70.6, 69.5, 68.8, 67.1, 33.2, 25.5, 20.7, 20.6, 20.6, 17.8, 17.1; HRMS (ESI) Calcd for $[M + Na]^+ = 583.1786$, found: $[M + Na]^+ = 583.1796$; IR (KBr) ν (cm^{-1}) = 3465, 1741, 1605, 1453, 1230, 1076.

*5.5.5. 1-(1-(5,8-Dihydroxy-1,4-naphthoquinone-2-yl)-4-methyl-3-pentenyloxy)-2,3,4-tri-O-acetyl-β-xylopyranoside (**5e**)*

Yield 86%, red solid, mp: 153–155 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 12.57 (s, 1H), 12.48 (s, 1H), 7.18 (m, 3H), 5.12 (m, 2H), 5.00 (m, 1H), 4.93 (m, 2H), 4.67 (d, $J = 6.4$ Hz, 1H),3.96 (dd, $J = 11.6$, 4.4 Hz), 3.27 (dd, $J = 10.4$, 9.6 Hz, 1H), 2.52 (m, 1H), 2.33 (m, 1H), 2.09 (s, 3H), 2.08 (s, 3H), 2.03 (s, 3H), 1.68 (s, 3H), 1.56 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 180.5, 179.2 170.5, 170.2, 169.8, 166.3, 165.8, 150.4, 135.8, 133.6, 132.9, 132.4, 118.6, 112.3, 112.0, 101.2, 75.4, 71.6, 71.2, 69.0, 62.4, 34.1, 26.3, 21.2, 21.2, 21.1, 18.4; HRMS (ESI) Calcd for $[M + Na]^+ = 569.1629$, found: $[M + Na]^+ = 569.1619$; IR (KBr) ν (cm^{-1}) = 3447, 1747, 1605, 1451, 1229, 1078, 1058.

*5.5.6. 1-(1-(5,8-Dihydroxy-1,4-naphthoquinone-2-yl)-4-methyl -3-pentenyloxy)-2,3,4-tri-O-acetyl-β-D-ribofuranoside (**5f**)*

Yield 51%, red solid, mp: 70–72 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ12.59 (s, 1H), 12.47 (s, 1H), 7.19 (m, 2H), 7.15 (m, 1H), 5.44 (t, $J = 3.2$ Hz, 1H), 5.13 (m, 1H), 5.08 (m, 1H), 5.05 (m, 1H), 4.99 (m, 2H), 3.81 (dd, $J = 3.2$, 12.4 Hz, 1H), 3.66 (dd, $J = 5.6$, 12.4 Hz, 1H), 2.53 (m, 1H), 2.41 (m, 1H), 2.12 (s, 3H), 2.09 (s, 3H), 2.07 (s, 3H), 1.68 (s, 3H), 1.57 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ179.2, 177.8, 169.8, 169.7, 169.5, 166.5, 166.0, 149.5, 135.8, 132.7, 132.6, 132.2, 117.9, 111.8, 111.5, 98.5, 74.0, 68.4, 66.5, 66.4, 61.6, 33.5, 25.7, 20.8, 20.8, 20.7, 18.0; HRMS (ESI) Calcd for $[M + Na]^+ = 569.1629$, found: $[M + Na]^+ = 569.1639$; IR (KBr) ν (cm^{-1}) = 3466, 1751, 1612, 1455, 1226, 1078.

*5.5.7. 1-(1-(5,8-Dihydroxy-1,4-naphthoquinone-2-yl)-4-methyl-3-pentenyloxy)-2,3,4-tri-O-acetyl-β-D-arabo-furanoside (**5g**)*

Yield 63%, red solid, mp: 52–53 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ12.59 (s, 1H), 12.43 (s, 1H), 7.17 (m, 2H), 7.04 (s, 1H), 5.24 (m, 2H), 5.16 (m, 2H), 5.01 (dd, $J = 3.2$, 8.8 Hz, 1H), 4.35 (d, $J = 6.4$ Hz1 H), 4.04 (dd, $J = 3.6$, 12.8 Hz, 1H), 3.58 (dd, $J = 1.6$, 12.8 Hz, 1H), 2.57 (m, 1H), 2.46 (m, 1H), 2.16 (s, 3H), 2.13 (s, 3H), 2.03 (s, 3H), 1.66 (s, 3H), 1.50 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 176.4, 175.6, 170.3, 170.0, 169.4, 169.1, 168.6, 148.3, 135.2, 133.5, 133.1, 132.1, 118.1, 111.8, 111.5, 98.9, 72.9, 70.0, 69.3, 67.3, 62.8, 34.3, 25.7, 21.0, 20.8, 20.6, 18.0; HRMS (ESI) Calcd for $[M + Na]^+ = 569.1629$, found: $[M + Na]^+ = 569.1635$; IR (KBr) ν (cm^{-1}) = 3472, 1749, 1611, 1455, 1223, 1061.

*5.5.8. 1-(1-(5,8-Dihydroxy-1,4-naphthoquinone-2-yl)-4-methyl-3-pentenyloxy)-2,3,4-tri-O-acetyl-β-L-arabofuranoside (**5h**)*

Yield 75%, red solid, mp: 127–129 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ12.57 (s, 1H), 12.50 (s, 1H), 7.25 (s, 1H), 7.19 (m, 2H), 5.22 (m, 2H), 5.12 (t, $J = 6.8$ Hz, 1H), 5.09 (dd, $J = 3.6$, 8.8 Hz, 1H), 4.97 (dd, $J = 4$, 5.6 Hz, 1H), 4.61 (d, $J = 6.4$ Hz, 1H), 3.86 (dd, $J = 3.6$, 12.8 Hz, 1H), 3.52 (d, $J = 12.8$ Hz, 1H), 2.53 (m, 1H), 2.36 (m, 1H), 2.13 (s, 3H), 2.10 (s, 3H), 2.07 (s, 3H), 1.68 (s, 3H), 1.55 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 180.5, 179.1, 170.3, 170.1, 169.3, 165.4, 164.9, 150.0, 135.4, 133.4, 132.2, 131.8, 118.1, 111.8, 111.6, 100.5, 74.6, 69.8, 69.2, 67.2, 62.7, 33.5, 25.8, 20.9, 20.8, 20.7, 18.0; HRMS (ESI) Calcd

for $[M+Na]^+ = 569.1629$, found: $[M+Na]^+ = 569.1614$; IR (KBr) ν (cm^{-1}) = 3468, 1747, 1609, 1454, 1226, 1061.

*5.5.9. 1-(1-(5,8-Dihydroxy-1,4-naphthoquinone-2-yl)-4-methyl-3-pentenyloxy)-2,3,6,2′,3′,4′,6′-hepta-O-acetyl-β-maltoside (**5i**)*

Yield 79%, red solid, mp: 72–74 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ12.59 (s, 1H), 12.43 (s, 1H), 7.17 (m, 3H), 5.52 (d, J = 5.2 Hz, 1H), 5.49 (d, J = 4 Hz, 1H), 5.36 (t, J = 10.0 Hz, 1H), 5.15 (m, 1H), 5.03 (t, J = 6.0 Hz, 1H), 4.98 (s, 2H), 4.84 (dd, J = 4.0, 10.4 Hz, 1H), 4.22 (m, 4H), 4.00 (m 2H), 3.80 (t, J = 6.4 Hz, 1H), 3.58 (d, J = 8.4 Hz, 1H), 2.36 (m, 1H), 2.28 (m, 1H), 2.09 (s, 3H), 2.08 (s, 3H), 2.06 (s, 3H), 2.04 (s, 3H), 2.02 (s, 3H), 2.00 (s, 3H), 1.98 (s, 3H), 1.66 (s, 3H), 1.56 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 176.7, 175.1, 170.5, 170.3, 170.1, 169.4, 169.2, 169.1, 168.5, 150.8, 135.5, 133.5, 133.1, 131.4, 121.9, 118.9, 111.8, 111.4, 96.9, 94.7, 73.0, 72.2, 70.2, 69.7, 68.6, 68.2, 68.2, 68.1, 67.6, 63.7, 61.7, 35.1, 25.6, 21.4, 20.8, 20.8, 20.7, 20.6, 20.6, 20.5, 17.9; HRMS (ESI) Calcd for $[M+Na]^+ = 929.2686$, found: $[M+Na]^+ = 929.2685$; IR (KBr) ν (cm^{-1}) = 3468, 1750, 1612, 1455, 1228, 1040.

*5.5.10. 1-(1-(5,8-Dihydroxy-1,4-naphthoquinone-2-yl)-4-methyl-3-pentenyloxy)-2,3,6,2′,3′,4′,6′-hepta-O-acetyl-β-cellubioside (**5j**)*

Yield 85%, red solid, mp: 161–163 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 12.59(s, 1H), 12.41 (s, 1H), 7.18 (m, 2H), 7.02 (s, 1H), 5.06 (m, 6H), 4.93 (m, 1H), 4.53 (m, 2H), 4.38 (m, 2H), 4.13 (dd, J = 4.8, 11.6 Hz, 1H), 4.05 (d, J = 12.4 Hz, 1H), 3.82 (t, J = 9.2 Hz, 1H), 3.66 (d, J = 8.8 Hz, 1H), 3.54 (dd, J = 3.6, 9.6 Hz, 1H), 2.53 (m, 1H), 2.40 (m, 1H), 2.16 (s, 3H), 2.12 (s, 3H), 2.09 (s, 3H), 2.02 (s, 6H), 2.01 (s, 3H), 1.98 (s, 3H), 1.66 (s, 3H), 1.51(s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 175.9, 175.0, 170.4, 170.1, 169.7, 169.6, 169.5, 169.2, 169.1, 168.9, 148.2, 135.0, 133.7, 133.3, 131.9, 118.3, 111.8, 111.5, 100.7, 99.0, 76.3, 73.7, 72.9, 72.7, 72.6, 71.9, 71.6, 71.5, 67.8, 61.6, 61.5, 34.2, 25.6, 20.8, 20.7, 20.6, 20.5, 17.9; HRMS (ESI) Calcd for $[M+Na]^+ = 929.2686$, found: $[M+Na]^+ = 929.2690$; IR (KBr) ν (cm^{-1}) = 3483, 1752, 1612, 1229, 1042.

*5.5.11. 1-(1-(5,8-Dihydroxy-1,4-naphthoquinone-2-yl)-4-methyl-3-pentenyloxy)-2,3,6,2′,3′,4′,6′-hepta-O-acetyl-β-lactoside (**5k**)*

Yield 81%, red solid, mp: 94–96 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 12.57 (s, 1H), 12.42 (s, 1H), 7.15 (m, 3H), 5.57 (d, J = 5.2 Hz, 1H), 5.45 (m, 1H), 5.34 (d, J = 2.8 Hz, 1H), 5.14 (m, 2H), 5.06 (dd, J = 3.6, 7.6 Hz, 1H), 4.96 (dd, J = 3.6, 10.4 Hz, 1H), 4.56 (d, J = 8 Hz, 1H), 4.26 (dd, J = 2.4, 4.4 Hz, 1H), 4.20 (dd, J = 2.0, 12.0 Hz, 1H), 4.07 (m, 3H), 3.90 (t, J = 6.8 Hz, 1H), 3.77 (m, 1H), 3.60 (d, J = 9.6 Hz, 1H), 2.36 (m, 1H), 2.29 (m, 1H), 2.14 (s, 3H), 2.08 (s, 3H), 2.07 (s, 3H), 2.02 (s, 3H), 2.00 (s, 3H), 1.94(s, 3H), 1.66 (s, 3H), 1.58(s, 3H), 1.55 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 177.5, 175.9, 170.6, 170.3, 170.2, 170.0, 169.3, 168.9, 168.4, 167.8, 150.7, 135.1, 133.2, 132.8, 132.0, 121.6, 119.2, 111.8, 111.6, 102.4, 96.9, 77.4, 73.2, 70.9, 70.8, 69.8, 68.8, 68.5, 67.1, 66.7, 63.4, 60.9, 35.3, 29.6, 25.7, 21.6, 20.8, 20.7, 20.6, 20.6, 20.5, 17.9; HRMS (ESI) Calcd for $[M+Na]^+ = 929.2986$, found: $[M+Na]^+ = 929.2949$; IR (KBr) ν (cm^{-1}) = 3466, 1751, 1612, 1227, 1050.

5.6. In vitro cytotoxic activity

5.6.1. Cell lines and cellular proliferation assay

MCF-7, HL60 and K562 cells were maintained in RPMI 1640 containing 10% fetal bovine serum. MCF-7/ADR, HL60/ADR and K562/ADR cells were grown in RPMI 1640 containing 10% fetal bovine serum and 1 μg/mL doxorubicin. All compounds were dissolved in DMSO with the stock concentration of 20 mM, and diluted with medium freshly before drug administration. MCF-7 and MCF-7/ADR cells were seeded into 96-well flat bottom plates at density of 3000 cells/well. Twenty-four h after seeding, each compound was added in triplicate. HL60 and HL60/ADR, K562 and K562/ADR cells were seeded at density of 8000 and 3000 cells/well respectively. Compound was added after seeding in triplicate. Cells in 96-well plate were incubated at 37 °C in a humidified atmosphere containing 5% CO_2. After 72 h, 20 μL MTT (5 mg/mL) was added into each well for 4 h incubation. After that, the supernatant was removed and 150 μL of dimethyl-sulphoxide (DMSO, Sigma) was added into each well in order to dissolve the blue-purple crystals of formazan. The absorbance was then measured using a model ELX800 Micro Plate Reader (Bio-Tek Instruments, Inc.) at 570 nm.

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