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Synthesis and biological evaluation of glyco-GA compounds as anticancer agents

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Abstract

A series of novel glyco-gambogic acid (GA) compounds were synthesized and evaluated for their *in vitro* anti-proliferative activity against human hepatocellular carcinoma (HCC) cells. All compounds showed much better aqueous solubility (0.92–1.89 mg/mL) than GA (0.013 mg/mL), and displayed potent inhibition on HCC cells (IC_{50}: 0.21–12.23 μmol/L) and little affects on non-tumor liver cells (IC_{50}: 42.56–86.43 μmol/L), suggesting that glyco-GA compounds selectively inhibit HCC proliferation, and may be promising candidates for further intensive study.

Keywords: Gambogic acid; Glyco-GA compounds; Anti-hepatocellular carcinoma activity

Gambogic acid (GA, **1**) is one of the major active ingredient of gamboge [1,2], which was used as a dye or folk medicine for an internal purgative and externally infected wounds [3]. Recent studies have demonstrated that GA possesses cytotoxicity against various human cancer cell lines, such as gastric carcinoma, hepatoma, and lung cancer cells, and displays inhibitory activity in tumor-bearing mouse models [4–7]. In addition, GA is not able to adversely affect the number of white blood cells (WBC) in blood and akaryote in marrow of rats [8]. Unfortunately, the aqueous solubility (0.013 mg/mL) of GA is very low, thus limiting its clinical application.

Structure-activity relationships (SARs) studies have shown that 30-carboxy group of GA can tolerate a variety of modifications with no or little effects on its bioactivity [9]. Additionally, a large variety of studies have demonstrated that introduction of carbohydrate moiety to a molecule usually improves its water solubility and cell penetrations, and enhances selectivity and bioactivity through intra/intercellular carbohydrate-protein interaction [10–13]. Accordingly, a series of novel glycol-derivatives of GA were therefore designed and synthesized by coupling various hydrophilic glycosyl groups to the 30-carboxyl of GA. After structural characterization, these target compounds were evaluated for their water solubility and inhibitory activity against HCC cell proliferation.

The starting material GA was obtained by isolation from gamboges and further purification, as described previously [1]. Coupling of 30-COOH of GA with *O*-acetylated glycosyl bromides, which were generated by treatment of corresponding mono- or di-saccharides with Ac_2O–CH_3COBr–MeOH [14], in the presence of K_2CO_3 and cetyl

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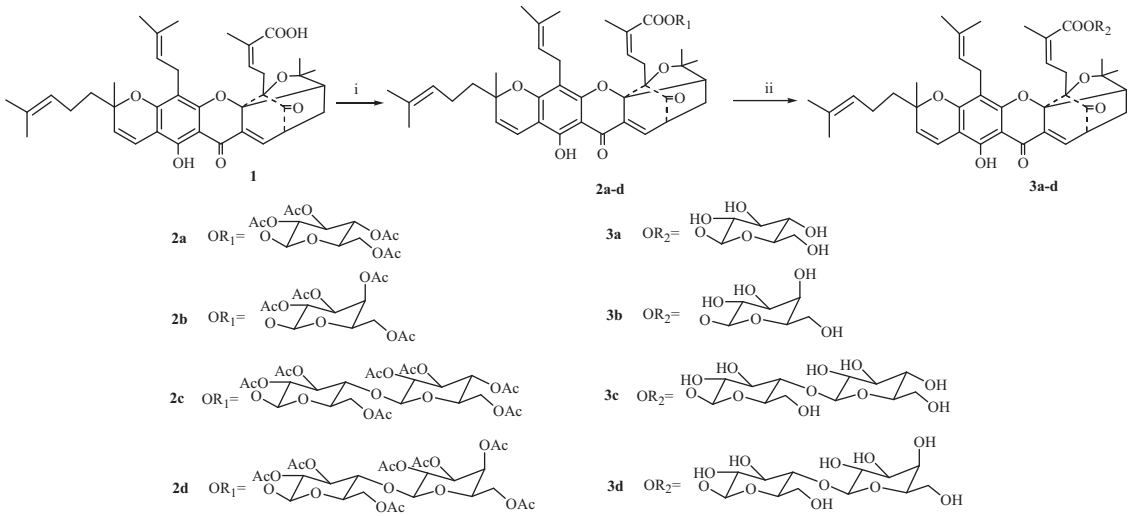

Scheme 1. The synthetic routes of **3a–d**. Regents and conditions: (i) Tetra-*O*-acetyl-*α*-D-glucopyranosyl bromide, tetra-*O*-acetyl-*α*-D-galactopyranosyl bromide, hepta-*O*-acetyl-*α*-D-maltosyl bromide or hepta-*O*-acetyl-*α*-D-lactosyl bromide, K_2CO_3, CTAB, H_2O–CH_2Cl_2, r.t., 48 h, in 70–80% yields; (ii) 25% NaOH, CH_3COCH_3, 0–10 °C, 20–30 min, in 50–72% yields.

trimethyl ammonium bromide (CTAB) in H_2O and CH_2Cl_2 gave acetylated esters **2a–d** in 70∼80% yields. Attempted deacetylation of **2a–d** with sodium methoxide in anhydrous methanol failed [15]. However, modified procedure using 25% sodium hydroxide in acetone successfully provided the target compounds **3a–d** in 50–72% yields, as shown in Scheme 1.

The structures of **3a–d** were characterized by spectra of IR, MS, ^{1}H NMR, and elemental analyses [16].

The aqueous solubility of glyco-GA compounds was determined as reported previously [17]. As shown in Table 1, all GA derivatives **3a–d** showed an increase in water solubility ranging from 0.92 to 1.89 mg/mL, which are 70- to 145-fold more soluble than the parent GA.

All target compounds were evaluated for their anti-proliferative effects on both human HCC cells and non-tumor cells *in vitro* by MTT assay [18], using GA as control (Table 2). The results indicated that anti-proliferative activity of **3a–d** against five HCC cell lines was largely comparable to or even stronger than that of GA. In sharp contrast, all of

Table 1
Solubility of the target compounds in water.

Compound	**3a**	**3b**	**3c**	**3d**	GA
Solubility (mg/mL)	0.92	0.95	1.78	1.89	0.013

Table 2
Anti-proliferative effects of GA and **3a–d** on both human HCC cells and normal liver cells HL-7702.

Compound	IC_{50} (μmol/L)[a]					
	HL-7702	BEL-7402	SMMC-7721	Bel-7404	QGY-7701	HepG2
GA	3.42	0.75	1.13	4.70	0.24	1.17
3a	86.43	1.12	2.43	3.02	0.21	2.01
3b	79.65	1.50	2.84	3.60	0.28	2.21
3c	44.83	10.68	10.41	7.85	4.89	9.86
3d	42.56	12.23	10.92	8.03	5.13	10.52

[a] IC_{50}: a concentration required to inhibit tumor cell proliferation by 50%. Data are expressed as the mean IC_{50} from the dose-response curves of at least three independent experiments.

them, especially for the most potent compounds **3a** and **3b**, displayed only little inhibition on proliferation of human normal liver cells HL-7702 (IC_{50}: 86.43 and 79.65 μmol/L *vs* 3.421 μmol/L), indicating that glyco-GA compounds selectively inhibited HCC cells.

The above data suggest that the introduction of glycosyl group(s) to the carboxyl group of GA may improve aqueous solubility and selective anti-proliferative effect of GA on human HCC cells. It was observed that the monosaccharide derivatives (**3a** and **3b**) had stronger inhibitory activity on human HCC cells and much lower inhibitory effects on non-liver cancer cell than disaccharide derivatives (**3c** and **3d**), probably due to that high levels of glucose transporter proteins and asialoglycoprotein receptors (ASGPR) are expressed in human HCC cells [11,19] and they may interact with those glucosyl and galactosyl derivatives by recognization and endocytosis, leading to preferable entry of **3a** and **3b** into HCC cells.

In summary, a new class of glyco-GA compounds were synthesized, and all of them exhibited better aqueous solubility than GA, and showed potent inhibitory effects on proliferation of human HCC cells. More importantly, they displayed little inhibition on non-HCC human liver cells. The most potent compounds **3a** and **3b** may be promising candidates for further intensive study. As a result, our novel findings provide a novel framework for the design of new glyco-GA compounds for the intervention of human HCC cells.

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[16] General procedure for the synthesis of the target compounds **3a–d**: compound **1** (628 mg, 1 mmol) was dissolved in the mixture of 15 mL CH_2Cl_2 and 20 mL H_2O in the presence of K_2CO_3 (207 mg, 1.5 mmol) and CTAB (91 mg, 0.25 mmol). The mixture was vigorously stirred and reacted with the corresponding O-acetylated glycosyl bromides (1.2 mmol) in several fractions at RT for 48 h. The organic layer was harvested and the remaining organic solvents in the aqueous layer were extracted with CH_2Cl_2 (10 mL × 3), dried over sodium sulfate, and concentrated in vacuo to obtain oil-like materials, which was subsequently purified by column chromatography using (PE/EtOAc = 3:1) to give pure **2a–c** in 70–80% yields. The resulting materials were dissolved in acetone on ice and its pH was adjusted to 9.0–10.0 with 25% sodium hydroxide. The deacetylation was monitored by TLC (1: 6, v/v, MeOH–CH_2Cl_2) and its pH was then adjusted to 5.0 with 10% HCl. After filtration, the filtrate was evaporated in vacuo and the resulting residue was extracted with EtOAc (10 mL × 3), dried over sodium sulfate, purified by column chromatography (EtOAc to MeOH–CH_2Cl_2 1:10, v/v) to give the title compounds (50–72%). Analytical data for compounds **3a–d**. Compound **3a**: yield 72%, yellow solid, mp: 134.8–135.0 °C. IR (KBr): 3304, 2930, 2866, 1735, 1715, 1626, 1596, 1456, 1070 cm^{-1}; MS (ESI, *m/z*): 789[M−H]$^-$; ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.93 (s, C_6-OH), 7.62 (d, 1H, *J* = 6.7 Hz, C_{10}H), 6.68 (d, 1H, *J* = 9.88 Hz, C_4H), 6.57 (s, 1H, C_{27}H), 5.66 (d, 1H, *J* = 7.82 Hz, sugar-H), 5.49 (d, 1H, *J* = 10 Hz, C_3H), 5.32 (m, 1H), 5.06 (m, 4H), 4.39 (s, 1H), 3.83 (m, 1H, sugar-H), 3.67 (d, 1H, *J* = 11.6 Hz, sugar-H), 3.39 (m, 2H, sugar-H), 3.20 (m, 4H), 2.96 (m, 1H), 2.90 (m, 1H), 2.52 (m, 1H), 2.30 (m, 1H), 2.08–2.01 (m, 2H), 2.00 (s, 3H), 1.90 (m, 1H), 1.81 (m, 1H), 1.75 (s, 3H), 1.68 (s, 3H), 1.58 (s, 3H), 1.43 (m, 1H), 1.38 (s, 3H), 1.31 (s, 3H), 1.27 (m, 1H), 1.17 (s, 3H). Anal. Calcd. for $C_{44}H_{54}O_{13}$: C 66.84, H 6.84; found: C 66.78, H 6.91. Compound **3b**: yield 70%, yellow solid, mp: 137.0–138.4.0 °C. IR (KBr): 3283, 2925, 2848, 1732, 1711, 1628, 1586, 1452, 1069 cm^{-1}; MS (ESI, *m/z*): 789[M−H]$^-$; ^{1}H NMR(500 MHz, DMSO-d_6): δ 11.93 (s, C_6-OH), 7.60 (d, 1H, *J* = 6.8 Hz, C_{10}H), 6.61 (d, 1H, *J* = 9.82 Hz, C_4H), 6.55 (s, 1H, C_{27}H), 5.61 (d, 1H, *J* = 8.12 Hz, sugar-H), 5.48 (d, 1H, *J* = 10 Hz, C_3H), 5.10 (m, 1H), 4.92 (m, 1H), 4.39 (s, 2H), 4.10 (s, 1H), 4,05 (s, 1H), 3.95 (m, 1H, sugar-H), 3.73 (m, 1H, sugar-H), 3.65(m, 1H, sugar-H), 3.47(m, 2H, sugar-H), 3.36 (m, 1H, sugar-H), 3.20 (m, 2H), 2.91 (m, 2H), 2.64 (m, 1H), 2.51 (d, 1H), 2.35(m, 1H), 2.19 (s, 3H), 2.01

(m, 1H), 1.90 (m, 1H), 1.83 (s, 3H), 1.75 (s, 3H), 1.68 (s, 3H), 1.57 (m, 1H), 1.45 (s, 3H), 1.42 (m, 1H), 1.38 (s, 3H), 1.29 (s, 3H), 1.26 (m, 1H), 1.14 (s, 3H). Anal. Calcd. for $C_{44}H_{54}O_{13}$: C 66.84, H 6.84; found: C 66.82, H 6.86. Compound **3c**: yield 54%, yellow solid, mp: 164.5–166.1 °C. IR (KBr): 3307, 2925, 2876, 1735, 1715, 1627, 1593, 1456, 1174, 1045 cm^{-1}; MS (ESI, *m/z*): 951 $[M-H]^-$; ^1H NMR (500 MHz, DMSO-d_6): δ 11.93 (s, C_6-OH), 7.62 (d, 1H, J = 6.7 Hz, C_{10}H), 6.62 (d, 1H, J = 10 Hz, C_4H), 6.41 (s, 1H, C_{27}H), 5.74 (d, 1H, J = 7.82 Hz, sugar-H), 5.58 (d, 1H, J = 10 Hz, C_3H), 5.42 (d, 1H, J = 9.1 Hz, sugar-H), 5.35 (d, 1H, J = 9.8 Hz, C_{37}H), 5.11 (d, 1H, J = 4.5 Hz), 5.06 (d, 1H, J = 5.5 Hz), 4.97 (d, 1H, J = 4.7 Hz), 4.80–4.89 (m, 3H), 4.39 (m, 1H), 4.26 (m, 1H, sugar-H), 4.18 (d, 1H, J = 11.6 Hz, sugar-H), 4.06 (m, 1H, sugar-H), 3.76 (m, 1H, sugar-H), 3.46 (m, 1H, sugar-H), 3.16 (m, 4H), 2.76 (m, 1H), 2.52 (m, 1H), 2.30 (m, 1H), 2.08–2.01 (m, 2H), 1.96 (s, 3H), 1.84 (m, 1H), 1.80 (m, 1H), 1.73 (s, 3H), 1.65 (s, 3H), 1.54 (s, 3H), 1.41 (m, 1H), 1.32 (s, 3H), 1.29 (s, 3H), 1.25 (m, 1H), 1.16 (s, 3H). Anal. Calcd. for $C_{50}H_{64}O_{18}$: C 63.03, H 6.72; found: C 62.91, H 6.85. Compound **3d**: yield 50%, yellow solid, mp: 174.2–175.5 °C. IR (KBr): 3306, 2932, 2878, 1732, 1713, 1627, 1594, 1457, 1072 cm^{-1}; MS (ESI, *m/z*): 951 $[M-H]^-$; ^1H NMR (500 MHz, DMSO-d_6): δ 11.95 (s, C_6-OH), 7.64 (d, 1H, J = 6.8 Hz, C_{10}H), 6.60 (d, 1H, J = 9.8 Hz, C_4H), 6.52 (s, 1H, C_{27}H), 5.49 (d, 1H, J = 8.12 Hz, sugar-H), 5.45 (d, 1H, J = 10 Hz, C_3H), 5.35 (d, 1H, J = 9.1 Hz, sugar-H), 5.32 (d, 1H, J = 9.8 Hz, C_{37}H), 5.12 (d, 1H, J = 4.3 Hz), 5.06 (d, 1H, J = 5.6 Hz), 4.83 (d, 1H, J = 4.8 Hz), 4.65 (m, 1H), 4.52 (d, 1H, J = 4.6 Hz), 4.47 (t, 1H, J = 5.8 Hz), 4.28 (m, 2H), 4.17 (m, 2H), 3.95 (m, 2H, sugar-H), 3.72 (m, 3H, sugar-H), 3.65(m, 2H, sugar-H), 3.47(m, 2H, sugar-H), 3.36 (m, 2H, sugar-H), 3.30 (m, 1H), 3.10 (m, 2H), 2.64 (m, 1H), 2.51 (d, 1H), 2.27(m, 1H), 2.16 (s, 3H), 2.01 (m, 1H), 1.96 (m, 1H), 1.83 (s, 3H), 1.77 (s, 3H), 1.69 (s, 3H), 1.58 (m, 1H), 1.46(s, 3H), 1.43(m, 1H), 1.37 (s, 3H), 1.29 (s, 3H), 1.26 (m, 1H), 1.14 (s, 3H). Anal. Calcd. for $C_{50}H_{64}O_{18}$: C 63.03, H 6.72; found: C 62.94, H 6.79.

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