Synthesis and antitumor properties of BQC-Glucuronide, a camptothecin prodrug for selective cancer activation: Supplementary Material

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Synthetic procedures

The synthesis is performed based on our published method for 10-hidroxycamptothecin[37]. Melting points were determined with a Kofler apparatus and are uncorrected. Reaction courses were routinely monitored by thin-layer chromatography (TLC) on silica gel pre-coated Durasil-25 UV254 Merck plates with detection under 254 nm UV lamp or heating. Nuclear magnetic resonance (^{1}H NMR) spectra were determined in DMSO-d_6 or $CDCl_3$ solution with a Bruker AC-200 spectrometer and chemical shifts are given in ppm with internal tetramethylsilane as a standard.

Preparative HPLC

BQC-G was dissolved in DMSO to 10 mg/mL, diluted 4 times with 10 mM KH_2PO_4, pH = 2.9 and injected into a reverse phase column packed with LichroPrep 40-63 μm. Separation was achieved using 30% ACN in 10 mM KH_2PO_4, pH 2.9 at 5 mL/min. Peaks were monitored by UV/Vis at 375 nm and by fluorescence at 375 nm excitation and 420 nm emission. The peak eluting at 180-200 min was diluted two-fold with water and reapplied to the column. After washing with 10% ACN in water, BQC-G was eluted with 50% ACN in water and evaporated under vacuum. DMSO was then added and evaporated to 1.5 mL. The product was stored at -80^{o}C.

Analytical SPE-HPLC

Samples were diluted with an equal volume of a mixture of acetonitrile (ACN): methanol: 0.5 M TCA 4:4:2 (v/v), vortex mixed to precipitate proteins and clarified at 15,000xg for 5 min at 4^{o}C. The supernatant was diluted in an equal volume of 0.1 M KH_2PO_4, pH 2.9 and injected into a SPE column. The SPE column was washed for 2.5 min with 5% ACN in 25 mM KH_2PO_4, pH 2.9 at 1 mL/min. A 6-port valve was employed to switch the mobile phase (30% ACN, 0.1 M KH_2PO_4, pH 2.9) in the reverse direction at 1.5 mL/min through the SPE column to transfer the analytes to the analytical column where they were separated and detected by fluorescence with excitation at 375 nm and emission at 575 nm for BQC, 460 nm for BQC-G, and 420 nm for 9ACG and 9AC.

Methyl 1,2,3,4-tetra-O-acetyl-beta-D-glucopyranuronate (10)

A mixture of D (+)-glucurono-3,6-lactone (**9**) (88 g, 500 mmol) and CH_3ONa (0.75 g, 13.9 mmol) in methanol (500 mL) was stirred at room temperature for 2 h. The reaction mixture was concentrated under reduced pressure to give a yellow-orange oily residue. To the residue was added acetic anhydride (340 mL). A solution of perchloric acid (1.5 mL) in acetic anhydride (10 mL) was then added to the mixture dropwise in an ice bath and stirred for 2 h. The resulting precipitate was recrystallized by methanol to give **10** (86 g, 46%) (Scheme I). mp 176^{o}C, ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.98 (t, J = 4.6 Hz, 9 H), 2.07 (s, 3 H), 3.62 (s, 3 H), 4.65 (d, J = 9.8 Hz, 1 H), 4.98 (q, J = 8.4 Hz, 2 H), 5.50 (t, J = 9.5 Hz, 1 H), 6.00 (d, J = 8.1 Hz, 1 H); ^{13}C NMR (50 MHz, DMSO-d_6) δ 21.0, 21.1, 21.2, 21.3, 53.5, 69.7, 70.7, 71.7, 72.3, 91.5, 167.8, 169.6, 169.9, 170.2, 170.3.

Methyl 1-alpha-bromo-1-deoxy-2,3,4-tri-O-acetyl –beta-D- glucopyranuronate (11)

A solution of **10** (1 g, 2.66 mmol) and $TiBr_4$ (1 g, 2.72 mmol) in CH_2Cl_2 (30 mL) was stirred at room temperature for 24 h. The mixture was washed with ice water (30 mL) and saturated aqueous $NaHCO_3$ solution (30 mL), dried over Na_2SO_4, and evaporated to dryness to give **11** (0.95 g, 90%), which was used directly in the next step without further purification.

Methyl 1-O-(2-nitro-4-formylphenyl)-2,3,4-tri-O-acetyl-beta-D- glucopyranuronate (12)

A suspension of **11** (0.95 g, 2.4 mmol), 4-hydroxy-3-nitrobenzaldehyde (0.44 g, 2.6 mmol) and Ag_2O (0.6 g, 2.6 mmol) in CH_3CN (50 mL) was stirred at room temperature for 4 h. AgBr was filtered out. The solvent was removed under reduced pressure to give a dark brown crude product, which was washed with methanol to give **12 (**0.95g; 82%). mp 180-182^oC, ^1H NMR (200 MHz, DMSO-d_6) δ 2.02 (d, J = 3.6 Hz, 9 H), 3.64 (s, 3 H), 4.81 (d, J = 9.6 Hz, 1 H), 5.16 (q, J = 7.7 Hz, 2 H), 5.48 (t, J = 9.3 Hz, 1 H), 5.95 (d, J = 7.5 Hz, 1 H), 7.65 (d, J = 8.7 Hz, 1 H), 8.23 (d, J = 6.9 Hz, 1 H), 8.45 (d, J = 1.7 Hz, 1 H), 9.99 (s, 1 H); ^{13}C NMR (50 MHz, DMSO-d_6) δ 21.0, 21.1, 21.2, 53.5, 69.3, 70.5, 71.3, 72.1, 98.1, 118.5, 127.1, 131.9, 135.6, 141.1, 152.9, 167.7, 169.6, 170.2, 170.4, 191.4 ; EI MS *m/z* 424 (M^+ - $OCOCH_3$). Anal. ($C_{20}H_{21}O_{13}N$): Calc. C, 49.55; H, 4.68; N, 2.89; Found. C, 49.46; H, 4.49; N, 2.73

Methyl 1-O-[2-nitro-4-(hydroxymethyl)phenyl]-2,3,4-tri-O-acetyl–beta-D-glucopyranuronate (13)

A mixture of **12** (0.9 g, 1.86 mmol), $NaBH_4$ (0.14 g, 3.72 mmol) and silica gel (2 g) in *i*-PrOH/$CHCl_3$ (1:5) (60 mL) was stirred at 0^oC for 1 h. The reaction was quenched with 1N HCl and filtered to remove silica gel. The organic layer was washed by water, then dried over

anhydrous Na_2SO_4 and evaporated under reduced pressure to give a residue which was crystallized with EtOH to give **13** (0.8 g, 89%). mp 167-168^oC, ^1H NMR (200 MHz, DMSO-d_6) δ 1.98 (d, J = 4.0 Hz, 9 H) 3.63 (s, 3 H), 4.49 (s, 2 H), 4.71 (d, J = 9.8 Hz, 1 H), 5.07 (t, J = 9.5 Hz, 2 H), 5.44 (t, J = 9.4 Hz, 2 H), 5.69 (d, J = 7.74 Hz, 1 H), 7.37 (d, J = 8.6 Hz, 1 H), 7.59 (d, J = 8.6 Hz, 1 H), 7.78 (s, 1 H); ^{13}C NMR (50 MHz, DMSO-d_6) δ 21.0, 21.1, 21.2, 53.5, 62.2, 69.6, 70.8, 71.7, 71.9, 98.9, 118.6, 123.2, 132.9, 139.4, 141.1, 147.8, 167.8, 169.6, 170.2, 170.4; FABMS *m/z* 426 (M^+ - $OCOCH_3$).

Methyl 1-O-[4-(methanesulfonyloxymethyl)-2-nitrophenyl]-2,3,4- tri-O-acetyl-beta-D-glucopyranuronate (14)

A solution of **13** (800 mg, 1.65 mmol) in CH_2Cl_2 (50 mL) was stirred with methanesulfonyl chloride (0.2 mL, 2.8 mmol) and triethylamine (0.4 mL, 2.97 mmol) at 0^oC for 1 h. The mixture was quenched with saturated aqueous $NaHCO_3$ solution, dried with anhydrous $MgSO_4$, and evaporated to dryness to give **14** (0.88 g, 95%) (Scheme II). mp 110-112^oC, ^1H NMR (200 MHz, DMSO-d_6) δ 2.00 (s, 9 H), 3.27 (s, 3 H), 3.63 (s, 3 H), 4.74 (d, J = 9.8 Hz, 1 H), 5.10 (q, J = 7.48 Hz, 2 H), 5.29 (s, 2 H), 5.46 (t, J = 9.4 Hz, 1 H), 5.77 (d, J = 7.7 Hz, 1 H), 7.47 (d, J = 8.4 Hz, 1 H), 7.79 (d, J = 8.6 Hz, 1 H), 8.02 (s, 1 H); FABMS *m/z* 562 (M^+ - 1).

10-[4-O-(Methyl-2,3,4-tri-O-acetyl-beta-D-glucopyranuronate)-3-nitro benzyloxy] 5,6-dihydro-4H-benzo[de]quinoline-camptothecin (15)

To a suspension of BQC (500 mg, 1.24 mmol) and Cs_2CO_3 (423 mg, 1.3 mmol) in anhydrous DMF (50 mL) was added compound **14** (0.836 g, 1.5 mmol). The mixture was stirred at room temperature for 2 h. The solvent was removed under vacuum before the solution was

added silica gel (5 g).The crude product was purified by column chromatography on silica gel (MeOH/$CHCl_3$ = 2:98) to give **15** (160 mg, 15%). ^{1}H NMR (200 MHz, DMSO-d_6) δ 0.87 (t, J = 7.1 Hz, 3 H, CH_3) 1.84-1.88 (m, 2 H, CH_2), 1.88-2.00 (m, 2H, CH_2), 2.01-2.03 (m, 9 H, CH_3), 2.93-2.94 (m, 2H, CH_2), 3.05-3.06 (m, 2H, CH_2), 3.63 (s, 3 H, OCH_3), 4.71 (d, J = 9.7 Hz, 1 H, sugar-H), 5.08-5.18 (m, 2 H, sugar-H), 5.25 (s, 2 H, CH_2), 5.31 (s, 2 H, CH_2), 5.40-5.52 (m, 1 H, sugar-H), 5.74 (d, J = 8.0 Hz, 1 H, sugar-H), 6.52 (s, 1 H, OH), 7.27 (s, 1 H, Ar-H), 7.35-7.71 (m, 3 H, Ar-H), 7.74-7.95 (m, 1 H, Ar-H), 8.10 (s, 2 H, Ar-H), 8.53 (s, 1 H, Ar-H)

10-[4-O-(Methyl-beta-D-glucopyranuronate)-3-nitrobenzyloxy] 5,6-dihydro- 4H-benzo[de]quinoline-camptothecin (16)

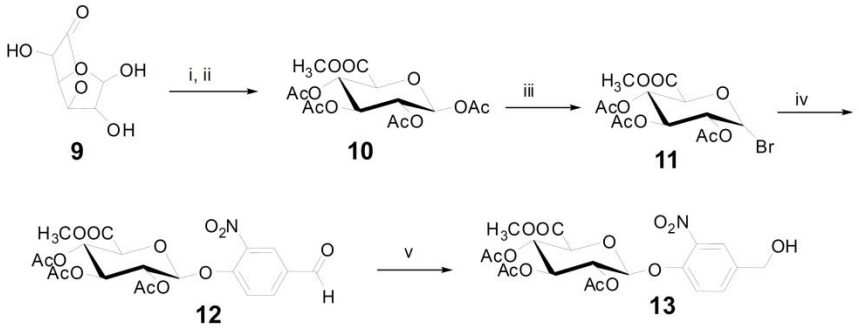
A suspension of **15** (160 mg, 0.18 mmol) and sodium methoxide (10 mg, 0.2 mmol) in anhydrous MeOH (30 mL) was stirred at room temperature for 2 h. The crude product was dissolved in dichloromethane / water (1:1) solution (20 ml). The dichloromethane layer was evaporated to dryness to give **16** (20 mg, 15%) (Scheme III). ^{1}H NMR (200 MHz, DMSO-d_6) δ 0.82 (t, J = 7.2 Hz, 3 H, CH_3), 1.75-1.77 (m, 2 H, CH_2), 1.88-1.91 (m, 2 H, CH_2), 2.72-2.82 (m, 2 H, CH_2), 2.95-3.01 (m, 2 H, CH_2), 3.32-3.48 (m, 3 H, sugar-H), 3.64 (s, 3 H, OCH_3), 4.13 (d, J = 8.9 Hz, 1 H, sugar-H), 5.26-5.53 (m, 9 H), 6.51 (s, 1 H), 7.27 (s, 1 H), 7.47-7.62 (m, 3 H), 7.81 (d, J = 8.3 Hz, 1 H), 8.08 (d, J = 8.6 Hz, 2 H), 8.53 (s, 1 H);

10-[4-O-(beta-D-glucopyranuronate)-3-nitrobenzyloxy] 5,6-dihydro- 4H-benzo[de]quinoline-camptothecin (8) (BQC-G)

The aqueous layer was acidified with 1 N HCl and purified by reverse phase column chromatography on silica gel (CH3CN/H2O = 1:5) to give **8** (35 mg, 27%). LCMS m/z

731.6(M+). ^{1}H NMR (200 MHz, DMSO-d_6) δ 0.88 (t, J = 7.1 Hz, 3H, CH_3); 1.86 (q, J = 7.2 Hz, 2H, CH_2); 1.95-2.05 (m, 2H, CH_2); 2.35-3.0 (m, 5H, CH_2 & sugar-H); 3.20-3.30 (m, 2H, CH_2); 3.75 (br s, 3H, OH); 5.14 (s, 2H, CH_2); 5.20 (d, J = 6.5 Hz, sugar-H); 5.31 (s, 2H, CH_2), 5.40 (s, 2H, CH_2), 5.50 (d, J =10 Hz, 1H, sugar-H), 7.26 (s, 1H, ArH); 7.49 (d, J = 8.8 Hz, 1H, ArH); 7.73-7.77 (m, 2H, ArH); 7.96-8.00 (m, 2H, ArH); ^{13}C NMR (200 MHz, DMSO-d_6) δ 8.2, 21.0, 31.2, 45.2, 53.8, 54.8, 72.8, 73.2, 75.1, 77.4, 96.0, 100.4, 117.0, 118.8, 121.6, 124.6, 126.0, 126.5, 128.0, 131.3, 133.7, 140.0, 140.2, 140.5, 144.0, 146.6, 149.6, 150.1, 150.5, 152.4, 157.2, 157.3, 172.9, 193.0.

Scheme I



Reagents:

i, Sodium methoxide, MeOH, rt, 1 h

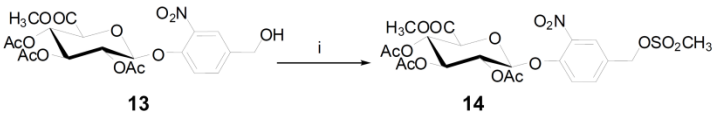
ii, $HClO_4$, acetic anhydride, rt,24 h, 46%

iii, $TiBr_4$, CH_2Cl_2,, rt, 24 h, 96%

iv, 4-hydroxy-3-nitrobenzaldehyde, Ag_2O, CH_3CN, rt, 24 h, 50-55%

v, $NaBH_4$, silica gel, *i*-PrOH, $CHCl_3$, 0 ^oC, 1 h, 70-90%

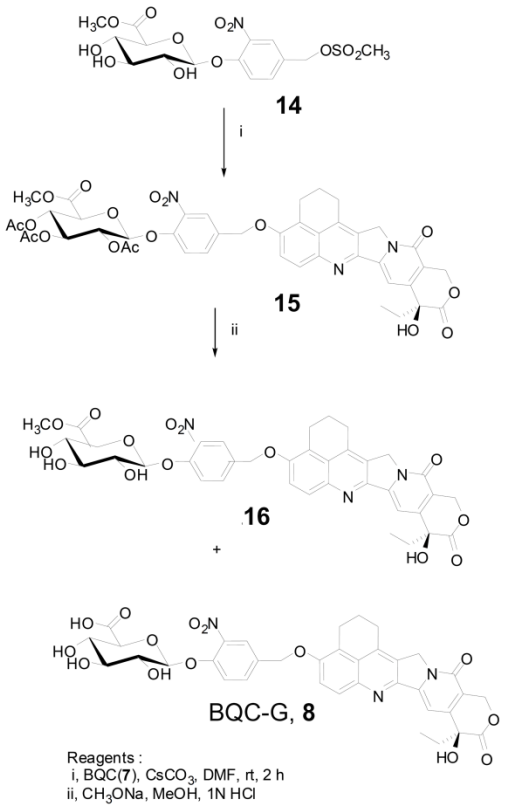
Scheme II



13 **14**

Reagents:
i, mesyl chloride, CH_2Cl_2, TEA, 0°C, 1 h

Scheme III


14
i
15
ii
16
+
BQC-G, 8
Reagents :
i, BQC(7), CsCO3, DMF, rt, 2 h
ii, CH3ONa, MeOH, 1N HCl