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Supplementary data

Bioreduction Activated Prodrugs of Camptothecin: Molecular Design, Synthesis, Activation Mechanism and Hypoxia Selective Cytotoxicity†

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Supporting Information

_p_-Nitrophenyl camptothecin carbonate CPT1

Camptothecin **(CPT**) derivatives **CPT1, CPT2** and **CPT3** were prepared according to the method reported recently by Pessah and coworkers.[7] In this study we employed modified synthetic procedures. **CPT** (338 mg, 0.97 mmol) and 4-nitrophenylchoroformate (408 mg, 2.0 mmol) were mixed in dichloromethane (30 mL) at 0 °C, followed by the addition of 4-dimethylaminopyridine (DMAP, 400 mg, 3.28 mmol). The resulting solution was stirred for 2 hours at room temperature and washed with 0.1 M HCl (4 × 10 mL). The organic layer was dried over sodium sulfate,

concentrated in vacuo to 10 mL, and then precipitated with hexane. The precipitated solid was recrystallized with dichloromethane-hexane to give **CPT1** (403 mg, 81%) as pale yellow powder. δ_H (270 MHz, $CDCl_3$) 1.05 (t, J = 7.4 Hz, 3 H), 2.15-2.39 (m, 2 H), 5.31 (s, 2 H), 5.40 (d, J = 17.5 Hz, 1 H), 5.70 (d, J = 16.5 Hz, 1 H), 6.92 (d, J = 8.9 Hz, 1 H), 7.39-7.45 (m, 2 H), 7.76-8.26 (m, 6 H), 8.43 (s, 1 H). δ_C (68 MHz, $CDCl_3$) 7.8, 31.9, 50.2, 67.1, 96.0, 115.6, 120.4, 121.6, 125.2, 126.1, 128.2, 128.3, 129.2, 131.1, 131.6, 144.9, 145.4, 146.3, 151.1, 154.9, 157.1, 166.6. FAB-HRMS (positive mode, NBA as matrix): *m/z* 514.1257 [MH^+], $C_{27}H_{20}N_3O_8$ requires 514.1250.

Camptothecin derivative CPT2

Mono-Boc-*N*,*N*'-dimethylethylenediamine (80 mg, 0.425 mmol) in dichloromethane (3 mL) was added dropwise to the solution of **CPT1** (100 mg, 0.195 mmol) in dichloromethane (10 mL). After stirred for 4 hours at room temperature, the reaction mixture was concentrated under reduced pressure. The residue was purified by PLC (ethyl acetate) to give **CPT2** (98 mg, 89%) as pale yellow powder. δ_H (270 MHz, $CDCl_3$) 0.93 (m, 3 H), 1.37 (m, 9 H), 2.02-2.27 (m, 2 H), 2.27-3.50(m, 10 H), 5.21(s, 2 H), 5.32 (d, J = 17.0 Hz, 1 H), 5.61 (d, J = 17.2 Hz, 1 H), 7.19 (m, 2 H), 7.51-8.32 (m, 4 H). δ_C (68 MHz, $CDCl_3$) 7.8, 28.5, 29.7, 35.5, 35.7, 47.3, 49.9, 67.1, 75.9, 97.7, 120.0, 125.2, 127.9, 128.1, 128.2, 128.5, 129.3, 130.7, 131.3, 145.7, 146.7, 148.5, 152.3, 152.7, 154.3, 155.7, 157.3, 167.9. FAB-HRMS (positive mode, NBA as matrix): *m/z* 563.2499 [MH^+], $C_{30}H_{35}N_4O_7$ requires 563.2506.

Camptothecin derivative CPT3

CPT2 (40 mg, 0.07 mmol) was treated by trifluoroacetic acid (1 mL) for 15 min at room

temperature. After removal of the solvent in vacuo, the residue was purified by PLC (ethyl acetate / methanol, 2 : 1) to give **CPT**3 (30 mg, 93%) as green yellow oil. δ_H (300 MHz, $CDCl_3$) 0.92 (m, 3 H), 2.02 (m, 2 H), 2.59-3.64 (m, 10 H), 5.30-5.66 (2 m, 4 H), 7.19 (m, 2 H), 7.44-8.30 (m, 4H). FAB-HRMS (positive mode, glycerol as matrix): *m/z* 463.1980 [MH^+], $C_{25}H_{27}N_4O_5$ requires 463.1981.

Camptothecin derivative CPT3a

Solution of mono-Boc-ethylenediamine (100 mg, 0.62 mmol) and **CPT1** (60 mg, 0.117 mmol) in mixed solvent of dimethylformamide (2mL) and dichloromethane (7 mL) was stirred at room temperature. After stirred for 1 hour, 30 mL of dichloromethane was added to the reaction mixture and kept under stirring for another 10 min. After removal of the precipitate by filtration, the filtrate was washed with 1M HCl (4 x15 mL) for 20 min, dried over sodium sulfate, and then concentrated under reduced pressure. The residue was purified by column chromatography (ethyl acetate / methanol, 4:1) to give **CPT3a** (31 mg, 60%) as pale yellow powder. δ_H (270 MHz, $CDCl_3$) 1.02 (m, 3 H), 2.04 (m, 4 H), 2.86-3.04 (2m, 4 H), 5.03-5.23 (2 m, 4 H), 7.20 (m, 2 H), 7.58-8.32 (m, 4 H). FAB-HRMS (positive mode, glycerol as matrix): *m/z* 435.1676 [MH^+], $C_{23}H_{23}N_4O_5$ requires 435.1668.

Camptothecin derivative CPT3b

CPT3b was prepared by the same method as **CPT3a**. **CPT1** (60 mg, 0.117 mmol) was treated by *N*-Boc-1,3-diaminopropane (100 mg, 0.57 mmol) in mixed solvent of dimethylformamide (2mL) and dichloromethane (7 mL) for 1 hour at room temperature. To the resulting reaction mixture was added additional 10 mL of dichloromethane, and

the precipitate was removed by filtration. The filtrate was then treated by 1M HCl (4 x 10 mL) for 20 min, and concentrated under reduced pressure. The residue was purified by column chromatography (ethyl acetate / methanol, 4:1) to give **CPT3b** (33 mg, 63%) as pale yellow powder. δ_H (270 MHz, $CDCl_3$) 1.03 (m, 3 H), 1.70-1.92 (2m, 6 H), 3.30 (t, J = 5.7 Hz, 2 H), 4.96 (m, 2 H), 5.28 (m, 2 H), 7.24 (m, 2 H), 7.61-8.37 (m, 4 H). FAB-HRMS (positive mode, glycerol as matrix): *m/z* 449.1813 [MH^+], $C_{24}H_{25}N_4O_5$ requires 449.1825.

Camptothecin derivative CPT3c

Solution of *N*-α-Boc-*L*-lysine (12 mg, 0.05 mmol), **CPT1** (25 mg, 0.05 mmol), DMAP (60 mg, 0.3 mmol) and triethylamine (0.03 mL, 0.4 mmol) in mixed solvent of acetonitrile (3 mL) and dichloromethane (DCM, 4 mL) was stirred. The reaction mixture was kept overnight under stirring at room temperature. After removal of the solvent by evaporation in vacuo, the residue was recrystallized with DCM (1 mL)-hexane (3 mL) for three times to give **CPT2c**. The resulting **CPT2c** was deprotected by TFA (0.5 mL) for 10 min. Evaporation and purification by PLC (ethyl acetate) gave **CPT3c** (12 mg, 46%) as pale yellow powder. δ_H (300 MHz, DMSO-d_6) 0.90 (t, J = 8.3 Hz, 3 H), 1.22-1.90 (4m, 8 H), 2.78 (t, J = 7.2 Hz, 2 H), 3.80 (t, J = 6.5 Hz, 1 H), 5.28 (s, 2 H), 5.4 (s, 2 H), 7.35-8.2 (3m, 6 H). δ_C (75 MHz, DMSO-d_6) 7.7, 21.4, 26.4, 29.5, 30.2, 50.2, 51.9, 65.2, 72.4, 96.7, 119.1, 127.7, 127.9, 128.5, 129.0, 129.8, 130.4, 131.5, 145.5, 147.9, 149.9, 156.8, 158.0, 160.5, 170.9, 172.5. FAB-HRMS (positive mode, NBA as matrix): *m/z* 521.2048 [$M+H]^+$, $C_{27}H_{29}N_4O_7$ requires 521.2036.

Camptothecin derivative CPT3d

Solution of 1-amino-1-deoxy-*β*-*D*-galactose (17.9 mg, 0.1 mmol), **CPT1** (51 mg, 0.1 mmol) and DMAP (30 mg, 0.25 mmol) in DMSO (2 mL) was stirred overnight at room temperature. Purification by PLC (ethyl acetate) gave **CPT3d** (37 mg, 66%) as pale yellow powder. δ_H (270 MHz, DMSO-d_6) 0.90 (m, 3 H), 1.93 (m, 2 H), 3.12-3.59 (m, 4 H), 4.60-4.85 (m, 2 H), 5.15-5.41 (m, 5 H), 7.48-8.12 (3m, 6 H). δ_C (75 MHz, DMSO-d_6) 7.5, 30.4, 50.2, 60.1, 60.4, 62.9, 68.1, 74.2, 74.5, 76.5, 95.3, 118.9, 127.7, 127.9, 128.5, 129.0, 129.8, 130.3, 131.3, 145.7, 146.2, 147.5, 147.9, 152.4, 154.4, 156.6, 167.8. FAB-HRMS (positive mode, NBA as matrix): *m/z* 554.1787 [MH^+], $C_{27}H_{28}N_3O_{10}$ requires 554.1775.

_E_-Ring opened analogue CPT3a'

Ethylenediamine (102 mg, 1.7 mmol) in dichloromethane (3 mL) was added dropwise to the solution of **CPT1** (89 mg, 0.17 mmol) in dichloromethane (7 mL) at 0 °C. After stirred for 15 min, the mixture was concentrated under reduced pressure. The residue was precipitated and washed with ethyl acetate (4 x 2 mL). Filtration gave **CPT3a'** (50 mg, 72 %) as pale yellow powder. δ_H (300 MHz, $CDCl_3$) 1.18 (m, 3 H), 1.92 (m, 2 H), 2.70-2.96 (2 m, 4 H), 5.23 (2 s, 4 H), 6.75 (m, 3 H), 8.00-8.12 (m, 3 H). FAB-HRMS (positive mode, glycerol as matrix): *m/z* 409.1880 [MH^+], $C_{22}H_{25}N_4O_4$ requires 409.1876.

_E_-ring opened analogue CPT3b'

1,3-Diaminopropane (14.8 mg, 0.2 mmol) in dichloromethane (2 mL) was added dropwise to the solution of **CPT1** (11 mg, 0.02 mmol) in dichloromethane (2 mL) at 0 °C. After stirred for 30 min, the mixture was concentrated under reduced pressure. The residue was precipitated and washed with ethyl acetate (4 x 0.5 mL). Filtration gave **CPT3b-1** (6 mg, 70 %) as pale yellow powder. δ_H (270 MHz, $CDCl_3$) 1.18 (m, 3 H),

1.82-1.92 (m, 4 H), 2.76 (m, 2 H), 3.28 (m, 2 H), 5.23 (2 s, 4 H), 6.73 (m, 3 H), 7.91-8.10 (m, 3H). FAB-HRMS (positive mode, glycerol as matrix): *m/z* 423.2043 [MH^+], $C_{23}H_{27}N_4O_4$ requires 423.2032.