Design, Synthesis, and Biological Evaluation of Camptothecin Loaded Biotinylated Cellulose Nanowhiskers as Anticancer Agents

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Abstract

Objective Using biotinylated cellulose nanowhiskers (CNWs), we designed and synthesized a Glutathione (GSH) sensitive- Camptothecin (CPT) prodrug for selective CPT delivery (compound **12**).

Methods CPT-biotin (compound 9), was synthesized by direct conjugation of CPT to the biotin via GSH sensitive linkage to evaluate the role of CNWs in compound **12**. The chemical structures of the synthesized prodrugs were confirmed by FT-IR, ^{1}H NMR, ^{13}C NMR, and ESI-MS, while the nanoparticles were characterized by DLS and TEM.

Results The in-vitro drug release assay demonstrated that only 18.6% of CPT was released from the nano conjugate under GSH stimulation at micromolar level (100 μM), while 83.1% accumulative release rate was achieved under GSH stimulation at millimolar level (10 mM). The in-vitro cytotoxicity assay (MTT assay) demonstrated that compound 9 showed higher inhibition ratios on biotin positive cells, MCF-7, and HepG2, and lower cytotoxicity on biotin negative, CHO. Compound **12** showed good activity against MCF-7, HepG2, and much lower cytotoxicity on CHO.

Conclusion This work demonstrates CPT-biotinylated cellulose nanowhiskers for selective chemotherapy and may have the potential to be used for cancer targeting.

Keywords Anticancer, camptothecin, cellulose nanowhiskers, biotin, glutathione.

Introduction

Cancer is a broad group of diseases that involves uncontrolled proliferation of abnormal cells that are capable of de-differentiation, invasion, and metastasis through the bloodstream or the lymphatic system.[1–5]

Camptothecins, like camptothecin (CPT, **1,** Figure 1) are regarded as one of the most promising anticancer drugs of the 21st century.[6] However, camptothecins have many challenges that need to be overcome. The problems include fast and pH-dependent hydrolysis of the lactone structure to give inactive carboxylate form, poor water solubility, and low and variable oral bioavailability.[7]

Different approaches are being investigated to modulate camptothecins' systemic delivery, such as the development of prodrugs, or semi-synthetic analogs such as topotecan (**2**) and irinotecan (**3**), which are used clinically for the treatment of colon and ovarian cancers, respectively. Another approach is the polymer conjugates such as amphiphilic PEG–CPT conjugates (**4**),[8,10] which improve the biological distribution, increase the body's retention times, reduce systemic toxicity, and improve the therapeutic efficacy.[8,11] Cellulose molecules with at least one dimension within the nanoscale (1–100 nm) are considered as a nanocellulose.[12] Nanocellulose can be divided into two main categories, cellulose nanowhiskers (CNWs), also known as cellulose nanocrystals (CNCs), and cellulose nanofibrils.[13,14] Among them, CNWs drew a lot of attention due to their properties like a high degree of crystallinity, smaller nanoparticle sizes, and better dispersion.[15] CNWs are considered interesting nanosize carriers for drug delivery because of their numerous advantages, like being biocompatible and not triggering an immune response.[16] CNWs have high hydrophilicity that impedes opsonin proteins' adsorption.[17] They are also suitable for different types of chemical linkages because the hydroxyl groups on the surface of CNWs can be easily modified to other chemical groups.[18]

In the current study, reduction-sensitive nanoparticles were designed from biotn- decorated CNWs- CPT with disulfide linkage. The presence of disulfide between the CNWs and CPT was suggested to release the active drug in response to the elevated intracellular GSH. Biotin has been used as targeting moiety with many anticancers to prevent the non-specific normal cells attack and to increase the uptake by the target cells. The *in vitro* drug release was studied at different GSH concentrations, and the cytotoxic effects of the nano conjugate on MCF7, HpeG2, and CHO cell lines were studied and compared with CPT prodrug without the nanocarrier.

Materials and Methods

Materials

Camptothecin, biotin, and 3,3'-dithiodipropionic acid were purchased from Beijing Yibai Biotechnology Co., Ltd. CNWs were purchased from the Process development center- The University of Maine U.S.A. Other chemicals were purchased

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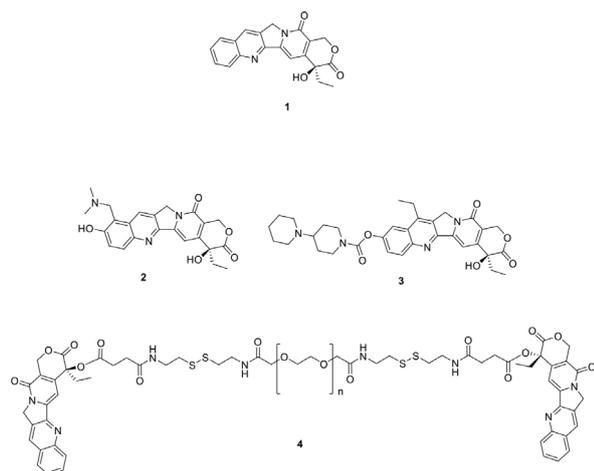
Fig. 1 **Chemical structures of CPT and -some CPT analogs and prodrugs.**

from Sigma–Aldrich. All chemicals are of analytical grade, and they were used as received without further purification.

Characterization of Compounds 5-9 and Compound 11

Melting points, Fourier transform infrared spectroscopy: FTIR, *NMR:* ^{1}H NMR and ^{13}C NMR spectra, and *Electrospray ionization mass spectrometry:* ESI-MS were performed for compound characterization.

Chemical Synthesis

The target compounds were synthesized by multi-step reactions, as shown in schemes 1&2.

Synthesis of Biotin Methyl Ester (Compound 5)

0.75 g (3.075 mmol) of biotin was suspended in 75 mL methanol, 0.5 g of the catalyst (Amberlite IR-120 resin) was added, and the suspension was stirred at room temperature for 24 h. After filtration and solvent's evaporation under reduced pressure, the title compound was obtained as a white powder, mp. 165-166ºC, yield 88%. ^{1}H NMR (500 MHz, DMSO-d6) 6.46 (1 H, s), 6.38 (1 H, s), 4.35 – 4.29 (1 H, m), 4.14 (1H, m), 3.59 (3 H, s), 3.12 (1 H, dd, J 4.9, 3.2), 2.83 (1 H, dd, J 12.4, 5.1), 2.59 (1 H, d, J 12.5), 2.31 (2 H, t, J 7.5), 1.68 – 1.42 (4 H, m), 1.35 (2 H, tt, J 14.3, 6.6). ^{13}C NMR (126 MHz, DMSO-d6) 173.78, 163.22, 61.52, 59.68, 55.81, 51.66, 40.33, 33.57, 28.48, 28.44, 24.95. ESI (MS) Calcd for $C_{11}H_{19}N_2O_3S$ (M+H)$^+$ 259.11, found 259.3.

Synthesis of Biotin Hydrazide (Compound 6)

258 mg (1 mol, 1.00 equiv) of compound **5** was dispersed in 4 ml of methanol, 0.5 mL, (10 mmol, 10.0 equiv) of hydrazine was added. The suspension was stirred for 24 h at room temperature. The solvent was removed under reduced pressure, and the title compound was obtained as a white powder after washing with chloroform, mp. 245-247ºC, yield 90%. ^{1}H NMR (500 MHz, DMSO-d6) 8.93 (1 H, s), 6.44 (1 H, s), 6.37 (1 H, s), 4.32 (1 H, dd), 4.15 (3 H, m), 3.10 (1 H, m), 2.83 (1 H, dd, J 12.4, 5.0), 2.59 (1 H, d, J 12.4), 2.02 (2 H, t, J 7.4), 1.67 – 1.41 (4 H, m), 1.31 (2 H, tt, J 14.3, 6.5). ^{13}C NMR (126 MHz, DMSO-d6) 172.02, 163.21, 61.52, 59.68, 55.89, 33.71,

28.70, 28.49, 25.70. MS (ESI) Calcd for $C_{10}H_{19}N_4O_2S$ (M+H)$^+$ 259.12, found 259.3.

Synthesis of 3,3'-Dithiodipropionic Anhydride (Compound 7)

3,3'-dithiodipropionic acid 2 g was dissolved in 5 mL of acetyl chloride in a round bottom flask and refluxed at 65ºC for 4 h until the solution was clear. After most of the solvent is removed under reduced pressure at 50ºC, iced ethyl ether was added to precipitate dithiodipropionic anhydride. After filtration and washing with petroleum ether, the title compound was obtained as a white powder, mp. 65-68ºC, yield 38%.

Synthesis of Camptothecin -3,3'-Dithiodipropionic Acid CPT-ss-COOH. (Compound 8)

Compound **7** (1.923 g, 10 mmol) and compound **1** (0.348 g, 1 mmol) were dissolved in pyridine (30 mL), a solution of DMAP (0.61 g, 5 mmol) in 10 mL of pyridine was added dropwise at 0 °C under nitrogen atmosphere. The reaction mixture was heated to 70 °C, and the reaction proceeded for 48 h. Then, the reaction solution was precipitated by using an excess of methanol, washed with dilute HCl, and dried to give the title compound as a yellow powder mp. 228-230ºC, yield 42%. ^{1}H NMR (500 MHz, DMSO-d6) 8.66 (1 H, s), 8.15 (1 H, d, J 8.5), 8.10 (1 H, d, J 8.2), 7.89 – 7.82 (1 H, m), 7.70 (1 H, t, J 7.5), 7.18 (1 H, s), 5.51 (2 H, s), 5.26 (2 H, s), 3.09 – 2.89 (6 H, m), 2.59 (2 H, t, J 7.0), 2.18 (2 H, qd, J 7.2, 3.8), 0.92 (3 H, t, J 7.4). ^{13}C NMR (126 MHz, DMSO-d_6) 173.43, 170.84, 167.60, 157.00, 152.76, 148.36, 146.41, 145.71, 131.97, 130.87, 130.19, 129.42, 128.95, 128.41, 128.15, 119.30, 95.71, 76.69, 66.76, 50.68, 34.37, 33.72, 33.62, 32.90, 30.69, 8.06. MS (ESI) Calcd for $C_{10}H_{19}N_4O_2S$ (2M+H)$^+$ 1081.21, found 1081.00.

Synthesis of Camptothecin-SS-Biotin Hydrazide (Compound 9)

To a stirred solution of compound **8** (540 mg, 1 mmol) in 10 mL of DMF, EDC (288 mg, 1.5 mmol) and NHS (172 mg, 1.5 mmol) were added. The solution was stirred at room temperature for 1 h to form NHS ester of compound **8**. Compound
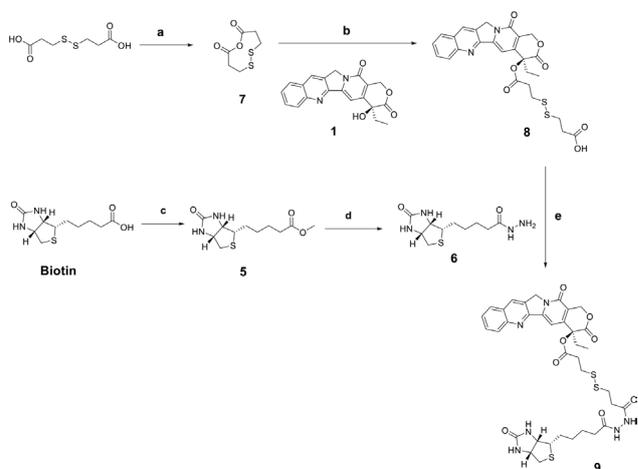
Scheme. 1 **Synthesis schematics of compound 9. Reagents and conditions: (a) acetyl chloride, 65ºC, 4 h, (38%); (b) DMAP, pyridine, 70°C, 48 h, (42%); (c) Amberlite IR 120 resin, methanol, rt, 24 h, 88%; (d) hydrazine, methanol, rt, 24 h, 90%; (e) EDC/ NHS, DMSO, rt, 48h, 72%.**

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6 (258 mg, 1 mmol) was added to the reaction solution, and the mixture was stirred at room temperature for 48 h. The product was precipitated by the addition of water (100 mL). The product was purified by washing with dilute HCl, and then dried to give the title compound as a white powder, mp. 234-236, yield 72%. ^{1}H NMR (500 MHz, DMSO-d6) 9.85 (1 H, d, J 2.1), 9.74 (1 H, d, J 2.1), 8.68 (1 H, s), 8.17 (1 H, d, J 8.5), 8.13 (1 H, d, J 8.2), 7.91 – 7.84 (1 H, m), 7.72 (1 H, t, J 7.6), 7.18 (1 H, s), 6.41 (1 H, s), 6.36 (1 H, s), 5.51 (2 H, s), 5.29 (2 H, s), 4.31 (1 H, m), 4.12 (1 H, m), 3.13 – 3.06 (1 H, m), 3.01-2.92 (6 H, m), 2.82 (1 H, dd, J 12.5, 5.1), 2.61 – 2.51 (3 H, m), 2.18 (2 H, qd, J 7.2, 3.8), 2.12 (2 H, t, J 7.4), 1.56 – 1.43 (4 H, m), 1.35 (2 H, tt, J 14.3, 6.5), 0.94 (3 H, t, J 7.4). ^{13}C NMR (126 MHz, DMSO-d_6) 171.35, 170.86, 169.41, 167.62, 163.17, 157.02, 152.80, 148.37, 146.43, 145.69, 132.02, 130.92, 130.25, 129.45, 128.99, 128.45, 128.19, 119.33, 95.70, 76.67, 66.78, 61.50, 59.66, 55.87, 50.71, 33.93, 33.65, 33.42, 33.39, 32.82, 30.72, 28.55, 28.48, 25.51, 8.06. MS (ESI) Calcd for $C_{10}H_{19}N_4O_2S$ (M+H)$^+$ 781.21, found 781.00.

Synthesis of Carboxylated CNWs (CNW-COOH, Compound 10)

1.02 g of CNWs were suspended in 100 ml of distilled water and sonicated for 5 min. 29.5 mg, 0.188 mmol of TEMPO, and 324 mg, 3.15 mmol NaBr were added to the suspension. Then, 3.15 mmol of NaOCl was added slowly to the cellulose suspension. The pH of the mixture was kept at 10-11 by using 0.5 M NaOH while stirring the suspension. After about 45 min, the oxidation was terminated by adding 2 mL of methanol. 0.5 M HCl was used to adjust the pH 7. The oxidized CNWs were dialyzed against deionized water for 48 h. The concentration of the suspension was determined gravimetrically.

Synthesis of Camptothecin -3,3'-Dithiodipropionic Hydrazide (Compound 11)

Compound **8** (540 mg, 1 mmol) was dissolved in 15 ml of DMF at room temperature. HOBt (162 mg, 1.2 mmol) and EDC (230 mg, 1.2 mmol) were added. The mixture was stirred at room temperature; the progress of the reaction was monitored by TLC. After 2 h when all the acid was converted to activated ester intermediates. The activated intermediate was then slowly added (by inverse addition) to a solution of hydrazine (2 mmol) and cyclohexene (0.05 mL) in DMF (10 mL) while the temperature was kept at 0-10 °C. The reaction was complete upon the completion of the addition. The product was precipitated by the addition 100 mL of cold distilled water. After filtration, the product was washed with distilled water and dried to get the title compound as a yellow powder, mp. 215- 218°C, yield 61%. ^{1}H NMR (500 MHz, DMSO-d_6) 9.06 (1 H, s), 8.62 (1 H, s), 8.13 (1 H, d, J 8.6), 8.07 (1 H, d, J 8.3), 7.89 – 7.81 (1 H, m), 7.67 (1 H, t, J 7.5), 7.17 (1 H, s), 5.50 (2 H, s), 5.22 (2 H, s), 4.36 (2 H, s), 3.02 – 2.89 (6 H, m), 2.51 (2 H, t, J 7.1), 2.17 (2 H, qd, J 7.2, 3.8), 0.93 (3 H, t, J 7.6). ^{13}C NMR (126 MHz, DMSO-d_6) 170.88, 169.91, 167.62, 156.97, 152.71, 148.32, 146.38, 145.71, 131.94, 130.86, 130.11, 129.40, 128.91, 128.36, 128.12, 119.31, 95.69, 76.67, 66.78, 50.63, 34.30, 33.65, 32.78, 30.72, 8.07. MS (ESI) Calcd for $C_{10}H_{19}N_4O_2S$ (M+H)$^+$ 555.13, found 555.10.

Synthesis of CPT-CNW-Biotin (Compound 12)

100 mL of compound **10** (0.74 W/V%) was flocculated by adding 6 mL of saturated NaCl, separated by centrifugation and rinsed (by redispersion, followed by centrifugation at 3000 rpm for 5 min) three times with 50 mL of acetone, then two times with 50 mL of DMF. The pellets were dispersed in 100 mL of DMF and subjected to 5 min of sonication. The concentration of CNW-COOH was determined gravimetrically (0.65% containing 0.75 mmol of COOH). 72 mg of EDC (0.37 mmol) and 43 mg of NHS (0.37 mmol) were added to activate half of the CNW-COOH. The suspension was stirred for 1 h at RT. 129 mg (0.5 mmol) of compound **6** was added, and the suspension was stirred for 24 h, and then the suspension was transferred to a dialysis tube and dialyzed against DMF for 24 h (3 times).

The suspension was transferred to a round bottom flask. 115 mg of EDC (0.6 mmol) and 69 mg of NHS (0.6 mmol) were added to activate the remaining CNW-COOH. The suspension was stirred for 1 h at RT. 333 mg (0.6 mol) of compound **11** was added, and the suspension was stirred for 24 h and then the suspension was transferred to a dialysis tube and dialyzed against DMF for 24 h (3 times) and then against DI water for 48 h.

Characterization of Nanoparticles

Conductometric Titration

The carboxyl content of compound **10** was determined using conductometric titration,[19] as shown in the titration curve (Figure 2).

Determination of Biotin and CPT Content

Elemental analysis was used to determine biotin content in compound **12** depending on the N%; LECO elemental analyzer was used. The amount of CPT was determined by UV-visible spectrophotometer at 372 nm using Carry 100 device.

Particle Size and Zeta Potential

Horiba SZ100 instrument was used to determine the particle size and Zeta potential.

Transmission Electron Microscopes (TEM)

After sonication in a water bath, very diluted suspensions of the nanoparticles (0.001 wt.%) were dropped on carbon film-covered copper grids. 2% uranyl acetate solution was
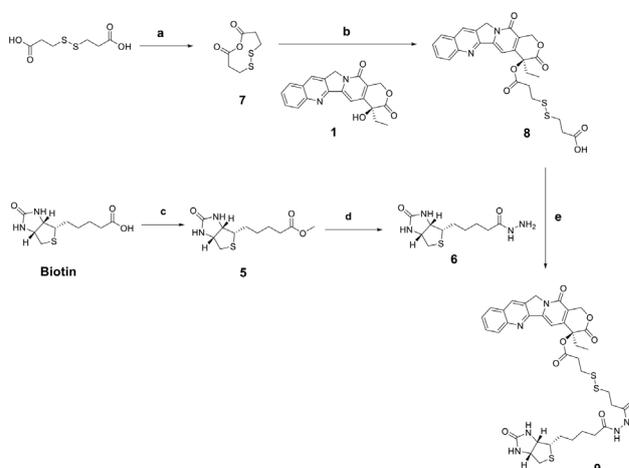
Scheme. 2 Synthesis schematics of compound 12. Reagents and conditions; (a) TEMPO, NaBr, NaClO, rt, 45 min; (b) HOBt, EDC, DMF, rt, 2 h; (c) inverse addition of HOBt ester to hydrazine, DMF, cyclohexene 0-10oC, 78%; (d) EDC, NHS, DMF, rt, 24 h.

used for staining the samples on the grid, and the drying was done at ambient conditions. Then, the samples were examined by TEM (PHILIPS Model: CM120).

In Vitro Drug Release Study

The CPT release experiments were carried out at 37°C. using two different media, acetate buffer (pH 5.8) with 10 mM GSH and phosphate buffer saline, pH 7.4 (FBS) with 100 µM GSH to simulate the different microenvironments in cancer cells and blood vessels, respectively.

In Vitro Cytotoxicity Assay

The in vitro cytotoxicity of compounds **9** and **12** was evaluated by MTT assay on biotin-positive cell lines, human breast cancer cell (MCF-7) and human hepatic carcinoma (HepG2), and biotin negative cell line, noncancerous Chinese hamster ovarian (CHO). MTT was performed to determine the cytotoxic effect of the samples at various concentrations. The results were given as the mean of three independent experiments and the IC_{50} values were then calculated.

Results

Chemical Synthesis of (5-9) and 11

Compound **5** was prepared by the esterification of biotin with methanol in the presence of high excess of methanol according to Fischer esterification using Amberlite IR120 resign as acid catalysis.[20] FT-IR showed the disappearance of carboxylic acid carbonyl stretching band (1700 cm^{-1}) and the appearance of new band at 1743 cm^{-1} due to ester carbonyl stretching. ^{1}H NMR of the compound **5** was characterized by the disappearance of COO$\underline{H}$ proton signal at 12.00 p.p.m. and the appearance of a new signal as an indication of biotin's successful esterification, the signal was related to COOC$\underline{H}_3$ protons at 3.59 p.p.m. as singlet.

Compound **6** was obtained by the hydrazinolysis of biotin methyl ester in methanol using an excess of hydrazine hydrate.[21] FT-IR showed the disappearance of ester carbonyl stretching band (1743 cm^{-1}) and the appearance of a new band at 1685 cm^{-1} due to hydrazide carbonyl stretching. ^{1}H NMR of compound **6** was characterized by the disappearance of COOC$\underline{H}_3$ protons at 3.59 p.p.m. and the appearance of new signals to indicate the success of hydrazinolysis of compound **5,** the signals were related to CON$\underline{H}$N$\underline{H}_2$ protons at 8.93 p.p.m. and 4.15 p.p.m. respectively both of them as a singlet.

Compound **7**, the FT-IR showed the new bands at 1793 cm^{-1} and 1739 cm^{-1} due to asymmetric and symmetric stretching vibration of anhydride carbonyl. Compound **8** was obtained by the esterification of the hydroxyl of compound **1** with the anhydride (compound **7**) **using DMAP as an acylating catalyst.**[8] FT-IR showed a new band at 1720 cm^{-1} due to the carboxylic acid carbonyl stretching. ^{1}H NMR of compound **8** was characterized by the disappearance of alcoholic proton signal at 6.53 p.p.m. and the appearance of new signals to indicate CPT esterification's success; the signals related to aliphatic protons at 3.00-2.59 p.p.m.

Compound **9** was obtained by the conjugation of compound **8** and compound **6** (biotin hydrazide) using EDC as a coupling agent.[22] FT-IR showed the disappearance of the carboxylic acid band at 1720 cm^{-1} due to amide formation. ^{1}H NMR of compound **9** was characterized by the disappearance of CON$\underline{H}$NH$_2$ signal at 4.15 p.p.m. and the appearance of new signals that were related to CON$\underline{H}$N$\underline{H}$CO protons at 9.85 p.p.m. and 9.74 p.p.m. both of them as a singlet. Compound **11** was obtained by the reaction of compound **8** with hydrazine using HOBt and EDC as coupling agents.[23] FT-IR showed the disappearance of the carboxylic acid band at 1720 cm^{-1} due to azide formation. ^{1}H NMR of compound **11** was characterized by the appearance of new signals to indicate the success of the reaction, the signals were related to CON$\underline{H}$N$\underline{H}_2$ protons at 9.06 p.p.m. and 4.36 p.p.m., respectively, both of them as a singlet.

Characterization of Nanoparticles

Conductometric Titration

The determination of carboxyl content of compound **10** was done using a method derived for the titration of cellulosic fibers by applying the following equation.

$$[COOH] = ((V*C\ NaOH) / m\ CNWs) - [OSO3H]\ in\ mmol\ kg^{-1}$$

Where V is the volume of NaOH (14.63 mL), C_{NaOH} is the NaOH concentration (0.01 mol/L), m CNWs is the weight of the sample (0.00015 kg), and [OSO$_3$H] was determined by elemental analysis and it was 175 mmole. By applying the equation, the [COOH] was calculated, and it was found to be 1150 mmole/kg.
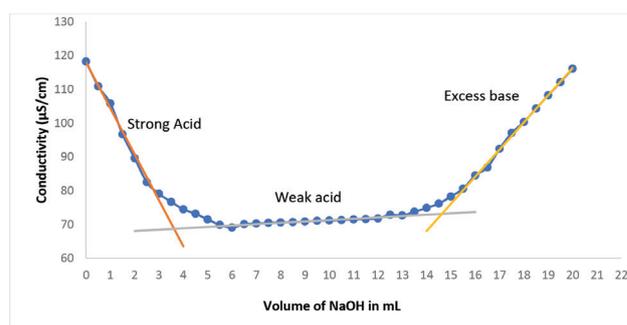
Fig. 2 **Conductometric titration of compound 10.**

Table 1: **Characterization data of CNWs and CNW conjugates.**

Sample	Particle size	PDI	Zeta potential	Biotin %	CPT %
CNWs	68.1±4.19	0.383±0.124	-34.33±1.59	–	–
Compound 10	81.23±0.3.53	0.462±0.052	-65.2±2.40	–	–
Compound 12	87.26±3.496	0.369±0.035	-45.16±1.20	8.67	5.6

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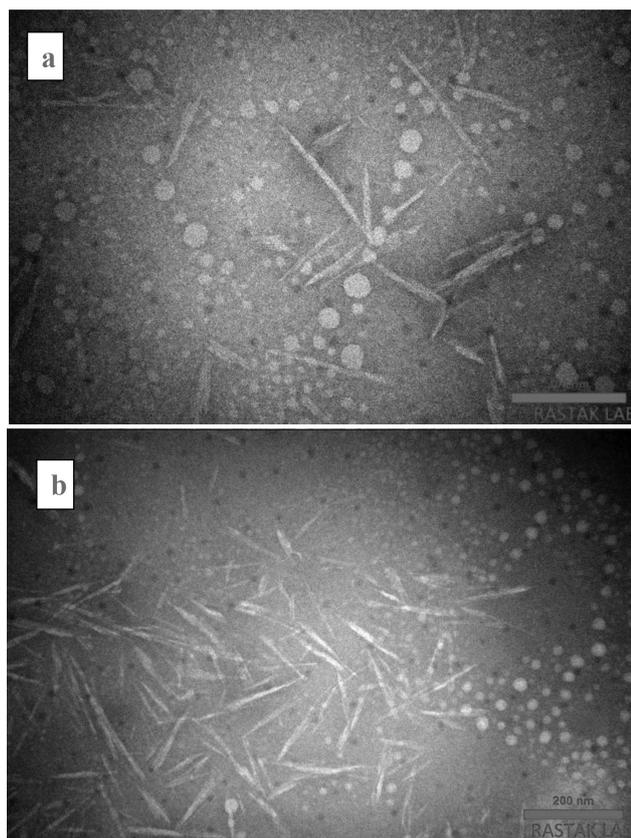
Fig. 3 **TEM images of (a) CNWs (b) compound 12.**

Scheme. 3 **CPT release mechanism from compound 12.**
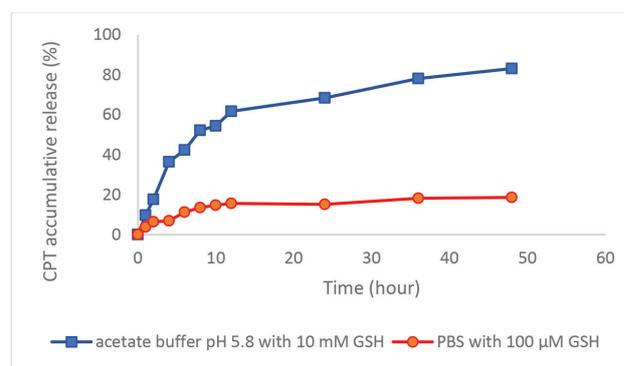
Fig. 4 **Accumulative release of CPT from compound 12 at different conditions.**

Determination of Biotin and CPT Content

The biotin and CPT contents of compound **12** were shown in Table 1.

Particle Size and Zeta Potential

The studies via DLS and zeta potential analysis revealed that the nanoparticles (CNWs, compounds **10**, and **12**) have a hydrodynamic radius between 68 and 87 nm and an overall negative surface charge, as shown in Table 1.

Transmission Electron Microscopes

Figure 3 shows the TEM images of CNWs, and compound **12**. No significant changes in the morphology or the degree of agglomeration were observed.

In Vitro Release Study

The disulfide linkage between the CNWs and CPT can be cleaved readily in the presence of a reducing agent such as GSH,[8] as shown in scheme 3.[24]

The accumulative release of CPT from compound **12** is only 18.6% in PBS with 100 μM GSH after 48 h, while the cumulative release in the acetate buffer medium pH 5.8 with 10 mM GSH is 83.1%, as shown in Figure 4.

In Vitro Cytotoxicity Assay

The anticancer activity of compound **9**, and compound **12** were examined in three cell lines with different expression levels of biotin receptor. MCF-7 and HepG2 cells overexpress biotin receptors on the surface, while biotin receptors were rarely expressed on CHO cells.[25] Figure 5a, b, and c show the viability of the cells after 72 h incubation. The half-maximal inhibitory concentration was determined to show the effectiveness of the tested compounds on growth inhibition of the three cell lines (Figure 5d).
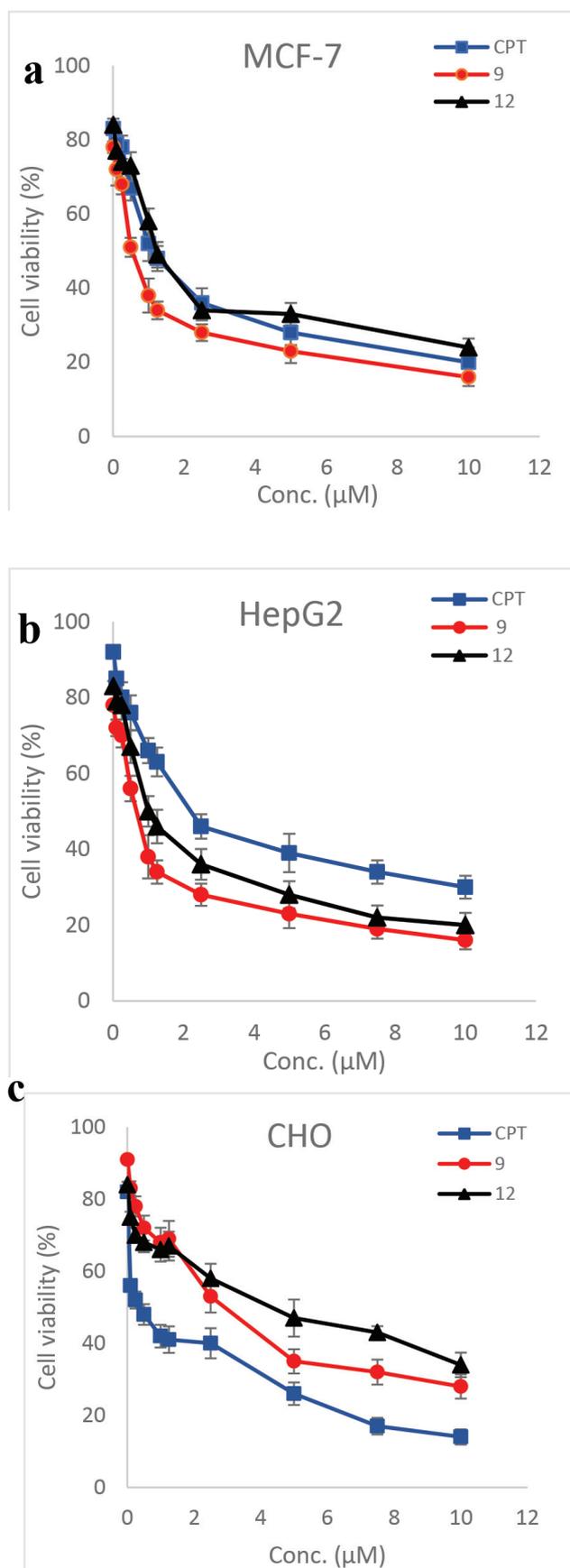
d

	MCF-7	HepG2	CHO
CPT	1.1 µM	2.17 µM	0.46 µM
9	0.51 µM	0.64 µM	3.24 µM
12	1.22 µM	1.04 µM	4.83 µM

Fig. 5 **Cytotoxicity of free CPT, compound 9, and compound 12 against (a) MCF-7, (b) HepG2, and (c) CHO cells after incubation for 72 h (n = 3) (d) IC50s of free 6-MP, compound 9, and compound 12 in MCF-7, HepG2, and CHO cells.**

Discussion

Chemical structure of the intermediates and the final compounds was confirmed using FT-IR, 1H NMR, 13C NMR, and ESI-MS. Characterization of nanoparticles was done using DLS and TEM. in Figure 4.

In vitro-release study results suggest that the release of CPT is accelerated in the cellular environment (especially tumors) with elevated GSH levels. In contrast, the release is minimum in the extracellular environment with a low GSH level.

Regarding the in vitro cytotoxicity, The IC_{50} of compound **9** is less than half or third of that of CPT in biotin positive cell lines, MCF-7 and HepG2, respectively, and about seven-folds higher than CPT for the biotin negative CHO cell line. For compound **12**, it showed comparable activity to CPT against MCF-7 and higher activity against HepG2 with IC_{50} about half that of CPT, and much lower activity against CHO with IC_{50} is about eleven times higher than that of CPT. The relatively small molecule compound **9** has a cellular uptake by passive diffusion beside the cell endocytosis; conversely, compound **12** as a nanoparticle, mainly internalized only by cells endocytosis. These results suggest that the uptake and the cytotoxicity of compounds **9** and compound **12** were higher for cell lines with biotin over-expression than for cells with low biotin expression, and they can potentially reduce side effects of CPT by selectively killing biotin-positive tumor cells

Conclusions

In the recent decade, there have been several CPT-macromolecule prodrugs to address CPT limitations. However, there is still an unmet need to develop different types of macromolecule prodrugs to reduce systemic toxicity and improve the therapeutic efficacy. In the present study, we synthesized two CPT prodrugs using a biotin receptor and a GSH sensitive disulfide linker for intracellular delivery of CPT. The chemical structures of the synthesized prodrugs were confirmed by FT-IR, ^{1}H NMR, ^{13}C NMR, and ESI-MS, while the nanoparticles were characterized by DLS and TEM. The release study showed that GSH could promote the drug release at the intracellular millimolar level compared with GSH at a micromolar level. MTT assay demonstrated that this system

had a higher inhibition ratio on biotin positive cells, MCF-7, and HepG2, and lower cytotoxicity on negative biotin cells, CHO. Therefore, these biotin-guided, GSH dependent prodrugs had the potential to be used for cancer targeting.

Acknowledgments

Conflicts of Interest Disclosure

There are no conflicts of interest.

Competing Interests

The authors declare that they have no competing interests.

Source of Funding

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