Engineering Camptothecin-Derived Norbornene Polymers for Theranostic Application

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Supporting Information

ABSTRACT: A multifunctional stimuli-responsive nanotheranostic agent provides huge benefits in nanomedicine by combining both the diagnostic agent and the drug molecule in a single system. This nanosystem is capable of doing multiple tasks, for example, diagnosis, drug delivery, and monitoring the therapeutic response. Hence, theranostic agents are expected to play a significant role in personalized medicine. Herein, a new class of nanotheranostic agents, Pnr-Cbt-Cpt-Pg-Bn, is proposed for the effective delivery of camptothecin. This new class of polymer has been functionalized with a superparamagnetic norbornene cobalt unit for its use in magnetic resonance imaging (MRI). The NMR one-dimensional image confirms the MRI capability of this nanotheranostic agent. This is further modified with the poly(ethylene glycol)—biotin moiety for biocompatibility and site-specificity. The uniqueness of the design is confirmed by an in vitro study where a greater uptake of the nanotheranostic agent is observed when compared with free drugs. Hence, this new class of copolymer shows improved potential as nanotheranostic agents in drug delivery.

INTRODUCTION

Progress in the development of different chemotherapeutic agents has produced an enormous opportunity to fight against cancer for the past 2 decades.1 Anthracycline, alkaloid, and inorganic metals are the front line compounds that are used in chemotherapy.2 However, these small molecules suffer from nonspecific binding and poor aqueous solubility that limit their use in chemotherapy.3 In recent years, nanocarriers have gained significant interest in nanomedicine.4 Nanocarriers, including micelles, vesicles, liposomes, dendrimers, and polymer—drug conjugates, show promising activity against various cancers because of their ability to reach specific targeted sites.5–8

Among the different classes of chemotherapeutic agents, alkaloids show significant promising activity toward various cancers.9 Camptothecin (CPT) is one of the alkaloid classes of chemotherapeutic agents, which is well-known for its anticancer activity toward various cancers via inhibiting the function of topoisomerase I, an essential enzyme for protein synthesis in the transcription process.9 This drug was first isolated by Wall and Wani from the bark of Camptotheca acuminata in 1960, and the anticancer therapeutic activity was well-explored by different research groups later on.10–12 However, despite its promising anticancer activity toward various cancers, this small molecule suffers from stability and aqueous solubility, which limit its use in conventional cancer therapy in the biological system, for example, through oral administration or intravenous injection.13 Several efforts have been made by different research groups to address the problems associated with this small molecule by making different functional derivatives, but none of them show any promising activity because of the lack of site-specificity that causes adverse side effects.3–5

Recently, a polymer-based nanotheranostic system has become an emerging class of compounds that simultaneously integrates therapy and diagnosis.14 Among different systems, the use of a polymer-based drug-delivery vehicle has become prominent over all other existing systems because of its pharmacokinetics and biodistribution profile via the enhanced permeability and retention (EPR) effect and the capability of maintaining the therapeutic concentration over a longer period of time.15 Hence, this theranostic medicine, which is capable of assistance in diagnosis and monitoring the therapeutic response, plays a significant role in the era of personalized medicine.

Among several diagnostic techniques, magnetic resonance imaging (MRI) has gained significant attention because of its noninvasive nature.16 This modern technique employs MRI contrast agents that help change the relaxation behavior of the targeted nuclei present in the tissues. Of the various relaxation mechanisms, transverse relaxation is frequently employed to exploit the advantages of contrast imaging.17 Toward this goal, different literature studies have reported different types of nanocarriers that carry the magnetic particles along with drug molecules, but the noncovalent attachment or the encapsulation of these inorganic magnetic particles leads to poor aqueous
solubility, which causes severe side effects. The poor aqueous solubility also causes the lower contrast efficiency because the water molecules have poor access to the magnetic core; hence, there is a pressing need to have a covalent-attached magnetic nanocarrier that can lead to a highly water-soluble nanotheranostic system.

To reduce the adverse cytotoxic effect of the chemotherapeutic agent, researchers have been trying to modify the system by incorporating different pendant functionalities into the prodrug system to make it more site-specific.3,4,6 Basically, target specificity deals with those kind of molecules that are required for a cell to grow. Now, for the rapid growth of a cancerous cell, high uptake value of some specific vitamins leads to the expression of receptors on the cancer cell surface in high amounts, when compared with a normal cell surface. Therefore, functionalization of this kind of molecules (folate, biotin, fructose) can guide the chemotherapeutic agent toward the cancer cells more site-specifically, which reduces the side effects.18−20

In this work, we report the development of a nanotheranostic agent capable of sustained delivery of CPT and MRI because of the presence of a superparamagnetic norbornene cobalt unit. The attachment of cobalt carbonyl to the acetylene functionalized norbornene by Nicholas reaction at the monomeric level. The NMR one-dimensional (1D) image shows a very prominent effect on the transverse relaxation of water molecules in micromolar concentration. This nanotheranostic agent is further modified by poly(ethylene glycol)−biotin (PEG−biotin), which helps the nanocarrier become site-specific.21 This highly water-soluble functional polymer nanocarrier is expected to be useful in theranostics.

■ RESULTS AND DISCUSSION

Toward the goal of making a norbornene-based theranostic prodrug, three different monomers have been designed (Mono 1−3) and synthesized as shown in Scheme 1. The formation of all monomers was confirmed by 1H NMR and 13C NMR spectroscopy (Figures S1−S14). To synthesize Mono 1, we first prepared the propargyl-attached norbornene (2), where the propargyl proton signal appeared. 1H NMR spectrum at δ = 2.1 ppm in CDCl3 (Figure S3) and the formation of the product was further confirmed by 13C NMR spectroscopy.
The attachment of cobalt carbonyl to the propargyl moiety was confirmed by NMR spectroscopy. In the $^1$H NMR spectrum, the shifting of the signal at $\delta = 6.0$ ppm from 2.1 ppm confirmed the attachment of cobalt carbonyl to the terminal alkyne group (Figure 1a)\textsuperscript{22,26}.

**REACTION SCHEME**

The $^{13}$C NMR spectrum clearly supported the formation of a product as a new peak arose at $\delta = 200$ ppm corresponding to Co−CO (Figure S5). The synthesis of Mono 2 was a two-step process, starting fromexo-norbornene anhydride. The anhydride was heated to reflux in toluene with 11-amino undecanoic acid for 12 h to get 3 as a pure white powder (Figures S6 and S7). The S-camptothecin was then reacted with 3 by using $N,N'$-dicyclohexylcarbodiimide (DCC) and 4-dimethylamino-pyridine (DMAP). The crude product was purified by column chromatography separation to get Mono 2 as pure (Figures 1b and S8). Toward the site-specific theranostic system, we synthesized norbornene functionalized with PEG−biotin (Mono 3). To attach the biotin moiety (Scheme 1), first, we prepared amine-terminated Nor-PEG (7). For that, we synthesized boc-protected glycin (4) (Scheme 1). Boc anhydride was used to block the amine group of the glycin (Figures S9 and S10). The boc-protected glycin (1.05 equiv) was further reacted with PEG in tetrahydrofuran (THF) (1 equiv) ($M_n = 650$ Da) in the presence of DCC and DMAP, which gave the boc-protected amine-terminated PEG (5). This molecule was precipitated in cold hexane three times to get a white sticky material as product (5), which was confirmed by $^1$H NMR spectroscopy (Figure S11). The free −OH group present at the end of the PEG motif of compound 5 was functionalized toexo-norbornene carboxylic acid by using DCC and DMAP (Scheme 1). The product was precipitated in cold hexane to get boc-protected Nor-PEG amine (6) (Figure S12). The deprotection of Nor-PEG-amine boc (6) was done by
that the polymerizations were well-controlled (Figures 2 and S22), resulting in a narrow polydispersity index (PDI), with a good yield (70–80%). After establishing the homopolymerization conditions for all monomers, triblock copolymerization was carried out by using Grubbs third generation catalyst (G-3) at room temperature in an anhydrous DCM solvent by the sequential addition of Mono 1–3 (Figure 3a,b). The polymerization was monitored by 1H NMR spectroscopy. The molecular weights of macroinitiator 1 (Mn = 6000, PDI = 1.03), macroinitiator 2 (Mn = 21 000, PDI = 1.12), and the final triblock copolymer (Pnr-Cbt-Cpt-Pg-Bn, Mn = 39 000, PDI = 1.35) were measured in gel permeation chromatography (GPC) by using polymethyl methacrylate standards (Figure 3c). The shifting of GPC traces clearly indicated the formation of triblock copolymer (Pnr-Cbt-Cpt-Pg-Bn) (Figure 3c). The formation of the copolymer was confirmed by 1H NMR spectroscopy (Figure S19).

Next, to prove the theranostic capability of the nanocarrier, an NMR 1D experiment of the nanocarrier (Pnr-Cbt-Cpt-Pg-Bn) was performed. All experiments were performed using a 500 MHz Avance-III Bruker spectrometer equipped with a linear gradient amplifier parallel to the static magnetic field. Three sample solutions were prepared for a comparative study. A 1:5 mixture of H2O and D2O in v/v ratio was used as the common solvent for all three sample solutions. The contrast agent was added to the solvent to obtain concentrations of 0, 0.1, and 0.5 mM. The first solution (without any contrast agent, i.e., the 0 mM solution) was used as a reference. The sample solutions were poured in 5 mm quartz NMR tubes to obtain the MRI images.

The pulse sequence used to establish the efficiency of the contrast agent is a spin-echo-based sequence. The sequence employs an acquisition under a linear gradient strength of 10 g/cm to obtain a T2-weighted 1D image of the sample. Figure 5a,b depict the pictorial representation of the pulse sequence.
and the Fourier spectrum. The echo period in the pulse sequence has been varied from 10 to 100 ms in steps to 10 ms. The Fourier transform of the acquired free induction decay collected under gradient yields a $T_2$-weighted 1D image of the sample solution for various echo periods owing to the spatial encoding. A series of spectra indicates the rapidity with which the signal decreases. For a given concentration, the resulting spectra are plotted in a contour diagram for a spatial extension of 2 mm of the sample. All processing was performed using MATLAB software. The intensity of the acquired signal has been plotted in a descending color-scale of red to blue, where blue indicates a stronger signal compared with that of red (Figure 5c).

Figure 3. (a) A toolbox comprising the monomers (Mono 1–3). (b) Polymerization scheme of Pnr-Cbt-Cpt-Pg-Bn. (c) Gel permeation chromatogram of triblock copolymer (Pnr-Cbt-Cpt-Pg-Bn). Macro initiator 1 (Nor-Cob) $M_n = 6000$ Da (PDI = 1.03) ($n = 12$), macro initiator 2 (Nor-Cob-Cpt) $M_n = 22000$ Da (PDI = 1.12) ($n = 19$), and final triblock copolymer (Pnr-Cbt-Cpt-Pg-Bn) $M_n = 39000$ Da, (PDI = 1.35) ($p = 15$).

A contour diagram in any of the subplot of Figure 5c shows how quickly the signal decays from high intensity (red) to low intensity (blue). For the blank solution (0 mM), the signal nearly vanishes near 80 ms (the beginning of blue region), whereas the same behavior is observed at 50 and 30 ms for the 0.1 and 0.5 mM solutions, respectively (Figure 5c). Thus, the contour diagram clearly indicates that the decay of the acquired signal strongly depends on the concentration of the nanotheranostic agent.

After confirming the MRI capability of the nanotheranostic agent (Pnr-Cbt-Cpt-Pg-Bn), experiments at the cellular level were performed and compared with free CPT and the theranostic agent without being modified with biotin (Pnr-
Cbt-Cpt-Pg (Scheme S2). For the in vitro cytotoxicity study, HeLa wt cells (human cervical cancer cell line) were maintained in minimum essential medium containing 10% fetal bovine serum, penicillium (100 U/mL), and streptomycin (100 μg/mL). It was incubated at 37 °C in a 5% CO₂ environment according to American Type Culture Collection recommendations. Cells were seeded in 96-well plates at a density of 1 × 10⁴ cells per well and grown for 24 h.

Cells were exposed with serial dilutions of various drug concentrations in the media (25–500 μg/mL) of Pnr-Cbt-Cpt-Pg-Bn and Pnr-Cbt-Cpt-Pg at 37 °C. The cytotoxicity of the nanoggregates Pnr-Cbt-Cpt-Pg and free CPT on HeLa wt cells were also assessed by the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyltetrazolium bromide (MTT) assay (5 mg/mL) in PBS. The free CPT was dissolved in 0.01% dimethyl sulfoxide (DMSO) in media. It is well-documented that because of the poor solubility of CPT, it is difficult to be applied in chemotherapy. Although different analogues of CPT have been synthesized by different research groups, cytotoxicity has been tested by dissolving the moieties exclusively in DMSO. However, DMSO by itself is toxic toward various cell lines and thus to living systems. Because of the hurdles, the PEG-modified CPT has only reached clinical trials. Thus, testing the therapeutic activity of a drug by dissolving it in DMSO alone will not provide the actual therapeutic benefits. Because HeLa wt cell lines are used to test the cytotoxicity of both the controls (free CPT and the nanocarriers), an already reported quantity of 0.01% DMSO was used, as it is not toxic to the cells at this quantity. Because of the presence of PEG, our nanocarrier is soluble in biological media. However, to show a more pronounced effect of the nanocarrier in comparison with the free CPT, the cytotoxicity experiment for CPT was carried out in 0.01% DMSO in media, whereas for the nanocarrier, the experiment was exclusively carried out in biological media (no DMSO). This can correlate the solubility factor as a nominal amount of DMSO was added and hence can provide insight into the usefulness of the aqueous solubility of chemotherapeutic agents. Even the water-soluble CPT analogue topotecan-HCl has been given approval for the treatment of cervical cancer.

The incubation time for each was 24 h. Although the cytotoxicity experiment for CPT was done in 0.01% DMSO in media, free CPT was still suffering from poor solubility. Hence, the undissolved part was filtered, and in vitro studies were done with the remaining solution. The concentration of free CPT was calculated from the amount of CPT soluble in the stock solution, and different concentrations of CPT solutions were prepared from the stock solution to perform the MTT assay. Further, to understand the cytotoxic nature of biotin-modified nanocarrier (Pnr-Cbt-Cpt-Pg-Bn) because of the enhanced internalization through receptor-mediated endocytosis, the PEG modified nanocarrier (Pnr-Cbt-Cpt-Pg) was used as a control molecule to compare the effects (Scheme S2).

A fresh 20 μL solution of MTT was added to each well, followed by incubation for 4 h in 5% CO₂ at 37 °C. The medium in each well was removed, and 100 μL of DMSO was added to each well and agitated for 15 min. The absorbance of the purple solution was measured at 525 nm by the enzyme-linked immunosorbent (immunoadsorbant) assay (ELISA) plate reader (BioTek Instrument—ELx 800). The killing efficiency was prominent in the case of Pnr-Cbt-Cpt-Pg-Bn compared with free CPT and Pnr-Cbt-Cpt-Pg (Figure 6a,b). This can be attributed to the mechanism of receptor-mediated endocytosis, as Pnr-Cbt-Cpt-Pg-Bn can be easily internalized by the cancer cell through a biotin receptor, which is missing in the case of Pnr-Cbt-Cpt-Pg and free CPT. In addition, the higher uptake of Pnr-Cbt-Cpt-Pg by the cancer cells in comparison with the free CPT can be attributed to the solubility factor.

To further investigate the biotin-assisted internalization, cellular uptake studies of Pnr-Cbt-Cpt-Pg-Bn were explored and compared with Pnr-Cbt-Cpt-Pg and free CPT (Figure 7). Pnr-Cbt-Cpt-Pg-Bn could rapidly enter owing to the incorporated biotin group, which showed an excellent cell-penetrating activity to promote cell internalization compared with Pnr-Cbt-Cpt-Pg (Figure 7), whereas free CPT suffering from aqueous solubility could not show a pronounced effect. This was further confirmed by flow cytometry analysis, which shows about a 2 times higher uptake of nanocarriers (Pnr-Cbt-Cpt-Pg-Bn) in HeLa wt cell lines compared with Pnr-Cbt-Cpt-Pg at the same concentration (Figure 8), whereas for free CPT, the mean intensity value is lower than that of both the nanocarriers Pnr-Cbt-Cpt-Pg-Bn and Pnr-Cbt-Cpt-Pg, which can be attributed to the solubility factor (Figure 8).
The lower uptake of Pnr-Cbt-Cpt-Pg to the cancer cell lines (HeLa wt) compared with Pnr-Cbt-Cpt-Pg-Bn can be attributed to the presence of a receptor on the cancer cell surface and not on the normal cell surface and that makes our system site-specific as the nanocarrier follows the receptor-mediated endocytosis (Figure 9). However, the free CPT, which is suffering from the solubility issues, shows the lowest uptake compared with nanocarriers (Pnr-Cbt-Cpt-Pg-Bn and Pnr-Cbt-Cpt-Pg) (Figure 8).

**CONCLUSIONS**

This paper describes the synthesis of a unique cell-internalizable stimuli-responsive nanotheranostic agent for the purpose of MRI and drug-delivery application. The well-shielded therapeutic agent CPT into the polymeric micelle shows excellent triggered release of CPT in response to intracellular pH that enhances the therapeutic efficacy as well as reduces the cytotoxic effect. The covalently bound paramagnetic cobalt block stabilized inside the core of the micelle shows excellent T₂ relaxation properties in very low concentration. Further, owing to the presence of the biotin moiety, the nanothernostic agent shows a higher cellular uptake compared with free CPT and Pnr-Cbt-Cpt-Pg, which is the crucial characteristic of a nanotheranostic agent in site-specific cancer therapy. Thus, this molecule is expected to play a crucial role in theranostics.

**EXPERIMENTAL SECTION**

Synthesis of Mono 1. In an anhydrous two-neck round bottomed flask, 2 (0.8 g, 0.001586 mol) was dissolved in anhydrous DCM. N₂ gas was passed through the solution, and the flask was kept in an ice bath. In another flask, cobalt carbonyl (1.1 g, 0.00317 mol) was dissolved in anhydrous DCM. This was added in a dropwise fashion to the solution containing 2. After that, the reaction mixture was stirred for 2 h in room temperature. After the completion of the reaction (monitored by TLC), the solvent was removed under vacuum. The product was recovered by precipitating it from pentane. The pure product was collected by column chromatography separation process. (SiO₂, DCM, acetone). Yield: 0.63 g (0.00142 mol, 90%). ¹H NMR (CDCl₃, 500 MHz) δ (ppm): 6.5 (s, 2H), 5.2 (s, 2H), 4.8 (s, 2H), 2.9 (s, 2H), 6.0 (s, 1H)
Synthesis of Mono 2. 1 (250 mg, 0.72 mmol) was dissolved in anhydrous dimethylformamide (DMF), and DCC (178 mg, 0.86 mmol) was added to it. The reaction mixture was stirred for 1 h of (S)-camptothecin (225 mg, 0.64 mmol) and DMAP (9 mg, 0.072 mmol) were added to it. The reaction mixture was stirred for 24 h. After the completion of the reaction (monitored by TLC), the solvent was evaporated to dryness. A pure product was obtained by the column chromatographic separation method in the DCM:MeOH medium. Yield = 268 mg (0.039 mmol, 55%) \(^1\)H NMR (DMSO-\(d_6\) 500 MHz) \(\delta\) (ppm): 8.7 (s, 1H), 8.1 (m, 1H), 7.8 (t, 1H), 7.7 (t, 1H), 7.3 (s, 1H), 6.5 (s, 1H), 6.25 (d, 2H), 5.42 (s, 2H), 5.28 (s, 2H), 3.9 (t, 2H), 3.08 (s, 2H), 2.67 (s, 2H), 2.2 (t, 2H), 1.2−1.7 (br s, 23H) (Figure 1b). \(^{13}\)C NMR (CDCl\(_3\) 500 MHz) \(\delta\) (ppm): 177, 172.5, 169.5, 156.8, 153.7, 152.5,
The formation of the product was also confirmed by 1H NMR spectroscopy (Figure S8). Synthesis of Mono 3. d-Biotin (0.1 g, 0.0004 mol) was dissolved in anhydrous DMSO, and DCC (0.126 g, 0.00061 mol) was added to it. The reaction mixture was stirred vigorously for 1 h. Then 11 (0.316 g, 0.00038 mol) was added to the reaction mixture along with the DMAP (3 mg, 0.0000226 mol). The reaction mixture was stirred vigorously for 24 h at room temperature. After the completion of the reaction, the reaction mixture was precipitated from cold diethyl ether. The precipitate was redissolved in the DCM:MeOH mixture and again reprecipitated in diethyl ether three times to get Mono 3. Yield: 260 mg (0.00024 mol, 60%). 1H NMR (DMSO-d$_6$, 500 MHz) δ (ppm): 1H NMR (DMSO-d$_6$, 500 MHz): 8.2 (d, 1H), 6.9 (d, 1H) 6.2 (d, 2H), 4.59 (t, 2H), 4.43–4.4 (m, 2H), 3.6 (s, broad), 2.9 (s, 2H), 2.19–2.28 (m, 2H), 2.30–2.33 (t, 2H), 2.0–2.07 (m, 2H), 1.86–1.94 (m, 2H), 1.50–1.56 (m, 2H), 1.2–1.16 (m, 2H) (Figure S14). The formation of the product was also confirmed by MALDI analysis (Figure S18).

Preparation of Grubbs’ Third Generation Catalyst. Freshly prepared Grubbs’ third generation catalyst23 was used for all polymerization reactions. The desired amount of Grubbs’ second generation catalyst (G-2) was placed in a glass vial. To this, 2-bromopyridine was added and stirred for 2 min. The immediate green coloration of the reaction mixture confirmed the formation of the catalyst. The product was precipitated from pentane. The whole reaction was carried out inside of the glove box under the nitrogen atmosphere. Polymerization Procedure. Polymerization was carried out by following previously reported literature.21–23 In general, a desired amount of Grubbs’ third generation catalyst was added to each vial containing three monomers (Mono 1–3). Polymerization was carried out inside of the glove box under the nitrogen atmosphere.

The catalyst (3.5 mg) was transferred to the vial containing Mono 1 (30 mg, 0.057 mmol) via a syringe. The reaction was allowed to stir for 10 min to complete polymerization. An aliquot of the sample was quenched with ethyl vinyl ether, precipitated in pentane, and taken for GPC analysis. GPC was done in THF (flow rate = 1 mL/min). The molecular weight of macro initiator 1 (Nor-Cob) was measured as $M_n = 6000$ Da (PDI = 1.04) by using polymethyl methacrylate standard. Then, the second monomer (Mono 2) (115 mg, 0.17 mmol) was added to the reaction vial after dissolving it in a minimum quantity of anhydrous CH$_2$Cl$_2$. The reaction mixture was stirred for 8 h, and an aliquot was taken for GPC analysis. The molecular weight of macro initiator 2 (Nor-Cob-Cpt) was measured to be $M_n = 18000$ Da (PDI = 1.13) by using polymethyl methacrylate standard. Finally, Mono 3 (1.5 g, 1.45 mmol) was added to the reaction vial and stirred until polymerization was completed. Then, the reaction mixture was quenched with ethyl vinyl ether (1 mL). An aliquot was taken for GPC analysis, and the remaining product was precipitated from pentane, dissolved again in THF, passed through neutral alumina to remove the catalyst, and precipitated again from pentane to get a pure triblock copolymer (Pnr-Cbt-Cpt-Pg-Bn). The molecular weight of the final triblock copolymer was measured as $M_n = 39000$ Da, PDI = 1.34 (Figure 3c). The formation of the copolymer (Pnr-Cbt-Cpt-Pg-Bn) was further confirmed by 1H NMR spectroscopy (Figure S19). After successful synthesis of Pnr-Cbt-Cpt-Pg-Bn, the THF solution of the copolymer was passed through a neutral alumina column, and the precipitation was done in diethyl ether three times. Then, the resulting polymer (Pnr-Cbt-Cpt-Pg-Bn) was dialyzed against the THF/water (3:1) mixture for 1 day by using a dialysis membrane (cut off $M_n = 3500$ Da).

ASSOCIATED CONTENT

S Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.7b00221.

Experimental section and NMR characterization of all monomers, TGA data of Pnr-Cbt-Cpt-Pg-Bn, IR data, MALDI, NMR, and GPC characterization of polymers, and percentage of attachment (PDF)

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Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Hurley, L. H. DNA and its processes as targets for cancer therapy. Nat. Rev. Cancer 2002, 2, 188–200.
(2) Banerjee, S.; Wang, Z.; Mohammad, M.; Sarkar, F. H.; Mohammad, R. M. Efficacy of Selected Natural Products as Therapeutic Agents against Cancer. J. Nat. Prod. 2008, 71, 492–496.
(3) Chabner, B. A.; Roberts, T. G., Jr. Chemotherapy and the war on cancer. Nat. Rev. Cancer 2005, 5, 65–72.
(4) Mura, S.; Nicolas, J.; Couvreur, P. Stimuli-responsive nanocarriers for drug delivery. Nat. Mater. 2013, 12, 991–1003.
(5) Saha, B.; Haldar, U.; De, P. Polymer-chlorambucil drug conjugates: A dynamic platform of anticancer drug delivery. Macromol. Rapid Commun. 2016, 37, 1015–1020.
(6) Peer, D.; Karp, J. M.; Hong, S.; Farokhzad, O. C.; Margalit, R.; Langer, R. Nanocarriers as an emerging platform for cancer therapy. Nat. Nanotechnol. 2007, 2, 751−760.
(7) Bhattacharya, S.; Ganivada, M. N.; Dinda, H.; Sarma, J. D.; Shunmugam, R. Biodegradable Copolymer for Stimuli-Responsive Sustained Release of Doxorubicin. ACS Omega 2016, 1, 108−117.
(8) Surnar, B.; Jayakannan, M. Stimuli-responsive poly(caprolactone) vesicles for dual drug delivery under the Gastrointestinal Tract. Biomacromolecules 2013, 14, 4377−4387.
(9) Hertzberg, R. P.; Carana, M. J.; Holden, K. G.; Jakas, D. R.; Gallagher, G.; Mattern, M. R.; Mong, S. M.; Bartus, J. O.; Johnson, R. K.; Kingsbury, W. D. Modification of the Hydroxylactone Ring of Camptothecin: Inhibition of Mammalian Topoisomerase I and Biological Activity. J. Med. Chem. 1989, 32, 715−720.
(10) Wall, M. E.; Wani, M. C.; Cook, C. E.; Palmer, K. H.; McPhail, A. T.; Sim, G. A. Plant Antitumor Agents. I. The Isolation and Structure of Camptothecin, a Novel Alkaloidal Leukemia and Tumor Inhibitor from Camptotheca acuminata. J. Am. Chem. Soc. 1966, 88, 3888−3890.
(11) Venditto, V. J.; Simanek, E. E. Cancer Therapies Utilizing the Camptothecins: A Review of the in Vivo Literature. Mol. Pharmacol. 2010, 7, 307−349.
(12) Pommier, Y. Topoisomerase I inhibitors: camptothecins and beyond. Nat. Rev. Cancer 2006, 6, 789−802.
(13) Dallavalle, S.; Ferrari, A.; Bisotti, B.; Merlino, L.; Penco, S.; Gallo, G.; Marzi, M.; Tinti, M. O.; Martinelli, R.; Pisano, C.; Carminati, P.; Carenini, N.; Beretta, G.; Pereg, P.; De Cesare, M.; Pratesi, G.; Zunino, F. Novel 7-Oxyniminoethyl Derivatives of Camptothecin with Potent in Vivo and in Vivo Antitumor Activity. J. Med. Chem. 2001, 44, 3264−3274.
(14) Sumer, B.; Gao, J. Theranostic nanomedicine for cancer. Nanomedicine 2008, 3, 137−140.
(15) Duncan, R. The dawning era of polymer therapeutics. Nat. Rev. Drug Discovery 2003, 2, 347.
(16) Ahrens, E. T.; Bulte, J. W. M. Tracking immune cells in vivo using magnetic resonance imaging. Nat. Rev. Immunol. 2013, 13, 755−763.
(17) Lee, H.; Lee, E.; Kim, D. K.; Jang, N. K.; Jeong, Y. Y.; Jon, S. Antifouling Polymer-Coated Superparamagnetic Iron Oxide Nanoparticles as Potential Magnetic Resonance Contrast Agents for in Vivo Cancer Imaging. J. Am. Chem. Soc. 2006, 128, 7383−7389.
(18) Goldstein, J. L.; Anderson, G. W.; Brown, M. S. Coated pits, coated vesicles, and receptor-mediated endocytosis. Nature 1979, 279, 679−685.
(19) Paulmurugan, P.; Bhethanabotla, R.; Mishra, K.; Devulapally, R.; Foygel, K.; Sekar, T. V.; Ananta, J. S.; Massoud, T. F.; Joy, A. Folate receptor-targeted polymeric micellar nanocarriers for delivery of orlistat as a repurposed drug against triple-negative breast cancer. Mol. Cancer Ther. 2016, 15, 221−231.
(20) Zhao, J.; Lai, H.; Lu, H.; Barner-Kowollik, C.; Stenzel, M. H.; Xiao, P. Fructose-Coated Nanodiamonds: Promising Platforms for Treatment of Human Breast Cancer. Biomacromolecules 2016, 17, 2946−2955.
(21) Chen, S.; Zhao, X.; Chen, J.; Chen, J.; Kuznetsova, L.; Wong, S. S.; Ojima, I. Mechanism-Based Tumor-Targeting Drug Delivery System. Validation of Efficient Vitamin Receptor-Mediated Endocytosis and Drug Release. Bioconjugate Chem. 2010, 21, 979−987.
(22) Al-Badri, Z. M.; Tew, G. N. Well-defined acetylene-functionalized oxanorbornene polymers and block copolymers. Macromolecules 2008, 41, 4173−4179.
(23) Louie, J.; Grubbs, R. H. Highly Active metathesis catalysts generated in situ from inexpensive and air-stable precursors. Angew. Chem., Int. Ed. 2001, 40, 247.
(24) Rao, N. V.; Kishore, A.; Sarkar, S.; Sarma, J. D.; Shunmugam, R. Norbornene-derived poly-d-lysine copolymers as quantum dot carriers for neuron growth. Biomacromolecules 2012, 13, 2933−2944.
(25) Mukherjee, S.; Sarma, J. D.; Shunmugam, R. pH-sensitive nanoaggregates for site-specific drug-delivery as well as cancer cell imaging. ACS Omega 2016, 1, 755−764.
(26) Mukherjee, S.; Patra, D.; Dinda, H.; Chakraborty, I.; Shashank, L.; Bhattacharyya, R.; Sarma, J. D.; Shunmugam, R. Super paramagnetic norbornene copolymer functionalized with biotin and doxorubicin: A potential unique site-Specific theranostic agent. Macromolecules 2016, 49, 2411−2418.
(27) Pramanik, P.; Halder, D.; Jana, S. S.; Ghosh, S. pH-triggered sustained drug delivery from a polymer micelle having the β-thiopropionate linkage. Macromol. Rapid Commun. 2016, 37, 1499−1506.
(28) Mienea, L. A.; Sessions, L. B.; Ericson, K. D.; Glueck, D. S.; Grubbs, R. B. Phenylethynylstyrrene–Cobalt Carbonyl block copolymer composites. Macromolecules 2004, 37, 8967−8972.
(29) Nie, Z.; Fava, D.; Kamacheva, E.; Zou, S.; Walker, G. C.; Rubinstein, M. Self-assembly of metal–polymer analogues of amphiphilic triblock copolymers. Nat. Mater. 2007, 6, 609−614.
(30) Surnar, B.; Jayakannan, M. Stimuli-responsive poly(caprolactone) vesicles for dual drug delivery under the gastrointestinal tract. Biomacromolecules 2013, 14, 4377−4387.
(31) Kenyon, B.; Kleinberg, R.; Staley, C.; Gubelin, G.; Morris, C. Nuclear Magnetic Resonance Imaging—Technology for the 21st Century. Oilfield Rev. 1995, 7, 19−33.
(32) Ren, W. X.; Han, J.; Uhm, S.; Jang, Y. J.; Kang, C.; Kim, J.-H.; Kim, J. S. Recent development of biotin conjugation in biological imaging, sensing, and target delivery. Chem. Commun. 2015, 51, 10403−10418.
(33) Homayak, C.; Anson, F.; Thayumanavan, S. Supramolecular Polymers in Nanomedicine. In Reference Module in Chemistry, Molecular Sciences and Chemical Engineering; Atwood, J. L., Ed.; Elsevier Inc, 2016.
(34) Kim, D.-K.; Ryu, D. H.; Lee, J. Y.; Lee, N.; Kim, Y.-W.; Kim, J.-S.; Chang, K.; Im, G.-J.; Kim, T.-K.; Choi, W.-S. Synthesis and biological evaluation of novel A-ring modified hexacyclic camptothecin analogues. J. Med. Chem. 2001, 44, 1594−1602.
(35) Svenson, S.; Wolfgang, M.; Hwang, J.; Ryan, J.; Eliasof, S. Preclinical to clinical development of the novel camptothecin nanopharmaceutical CRLX101. J. Controlled Release 2011, 153, 49−55.
(36) Warnecke, A.; Kretzsch, P. Maleimide-oligo(ethylene glycol) derivatives of camptothecin as albumin-binding prodrugs: Synthesis and antitumor efficacy. Bioconjugate Chem. 2003, 14, 377−387.
(37) Malinin, T. I.; Perry, V. P. Toxicity of dimethyl sulfoxide on HeLa cells. Cryobiology 1967, 4, 90−96.
(38) Karthik, S.; Puvvada, N.; Kumar, B. N. P.; Rajput, S.; Pathak, A.; Mandal, M.; Singh, N. D. P. Photosensitive coumarin-tethered multifunctional magnetic nanoparticles for release of anticancer drug. ACS Appl. Mater. Interfaces 2013, 5, 5232−5238.
(39) Beauchamp, L. M.; Orr, G. F.; de Miranda, P.; Bumette, T.; Kretinsky, T. A. Amino Acid Ester Prodrugs of Acyclovir. Antiviral Chem. Chemother. 1992, 3, 157−164.
(40) Rao, N. V.; Dinda, H.; Venu, P.; Sarma, J. D.; Shunmugam, R. RSC Adv. 2014, 4, 45625.
(41) Saldani, A.; Kutty, S. Plant-derived compounds in clinical trials. Drug Discovery Today 2008, 13, 161−171.