Safe approaches for camptothecin delivery: structural analogues and nanomedicines

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Abstract

Twenty-(S)-Camptothecin is a strongly cytotoxic molecule with excellent antitumor activity over a wide spectrum of human cancers. However, the direct formulation is limited by its poor water solubility, low plasmatic stability and severe toxicity, which currently limits its clinical use. As a consequence, two strategies have been developed in order to achieve safe and efficient delivery of camptothecin to target cells: structural analogues and nanomedicines. In this review we summarize recent advances in the design, synthesis and development of camptothecin molecular derivatives and supramolecular vehicles, following a systematic classification according to structure-activity reationships (structural analogues) or chemical nature (nanomedicines). A series of organic, inorganic and hybrid materials are presented as nanoplatforms to overcome camptothecin restrictions in administration, biodistribution, pharmacokinetics and toxicity. Nanocarriers which respond to a variety of stimuli endogenously (e.g., pH, redox potential, enzyme activity) or exogenously (e.g., magnetic field, light, temperature, ultrasound) seem the best positioned therapeutic materials for optimal spatial and temporal control over drug release. The main goal of this review is to be used as a source of relevant literature for othersinterested in the field of camptothecin-based therapeutics.To this end, final remarks on the most important formulations currently under clinical trial are provided.

Keywords: camptothecin analogues; camptothecin nanomedicines; structure-activity relationship; stimuli-responsive; clinical trials

Contents

1. Introduction

Camptothecin (CPT) is a water insoluble, natural pentacyclic alkaloid isolated from the oriental tree *Camptotheca accuminata* by Wall et al. in 1966 [1]. CPT is well-known for its antitumor activity against a wide spectrum of human cancers [2-8]. In addition, a variety of biological activities, such as pesticidal [9-10], antipsoriasis [11-12], antiparasitic [13], antifungal [14], antimicrobial [15] and antiviral [16-17] has been described for this compound. Certainly, several researchers have reported that CPT inhibits a cellular enzyme DNA topoisomerase I and induces apoptosis in various cancer cells. [18-19]

CPT is a planar pentacyclic quinoline that includes 3 rings of pyrrolo- (3,4-β)- quinoline part (A, B, and C rings), an unsaturated pyridone moiety in ring D and a α-hydroxy lactone ring (E ring), containing one chiral center with (S)-configuration (Fig. 1) [20-21]. It is worth noting that the isomer 20(R) hydroxyl has little activity while the isomer 20(S) is between 10 and 100 fold more active [22]. Also, it is known that E-ring exists in equilibrium between the lactone form (closed ring, not water insoluble) and the carboxylate form (open ring, water soluble). In acidic pH the lactone predominates which, according to different clinical trials and structure-activities studies, is the only therapeutic molecule.

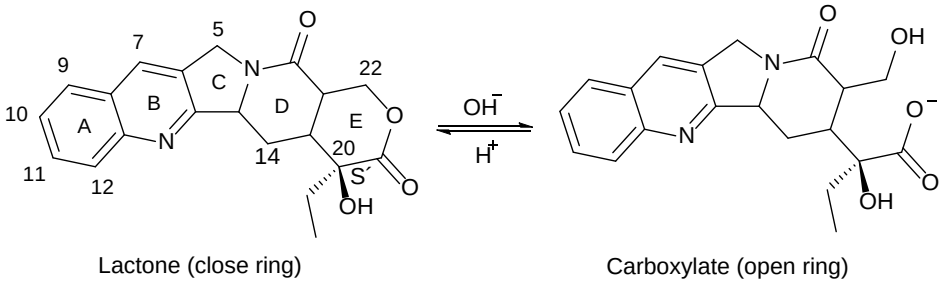


Fig. 1. CPT equilibrium between lactone (active form) and carboxylate (inactive form)

However, at physiological pH, the lactone ring is converted into the carboxylate, much less active, which predominates at neutral and alkaline pH [23-24].

Unfortunately, CPT presents some major limitations with regards to therapeutic application, like poor water solubility and the rapid lactone ring hydrolysis at physiological pH, which gives rise to the inactive carboxylate form [25]. In addition, CPT is extremely insoluble in organic compounds except for dimethyl sulfoxide, in which it shows moderate solubility [26]. Due to CPT insolubility in biocompatible solvents, it is very difficult to apply conventional drug administration routes, including oral, intravenous or intramuscular injection, to distribute this compound throughout the body [27]. Furthermore, there are other negatives aspects that limit the use of CPT in clinical trials: pronounced loss of activity due to lactone-ring hydrolysis, reversibility of drug-target interaction and severe toxicity, including hemorrhagic cystitis and myelotoxicity [28]. All these drawbacks have precluded its clinical application, making necessary to develop alternative compounds and structures for CPT use in humans.

In an effort to improve CPT solubility and pharmacokinetics structural derivatives as topotecan and irinotecan have been developed [29-30] (see Table 1). Unfortunately, as it will be reported later on, CPT quinoline structure modification usually brings out a dramatic drop of cytotoxic activity, which results one order or even lower than pristine drug, strongly limiting the therapeutic use.

To overcome these issues two strategies have been pointed out for safety and efficient CPT delivery to target cells: i) structural analogues, in which the CPT molecule is chemically modified for increased solubility and stability in biological fluids; and ii) nanomedicines, wherein CPT is incorporated by physico-chemical methods to nanoparticles which act as stable carriers for drug delivery. In this review, we summarize the most advanced and recent achievements in the design, synthesis and development of CPT analogues and nanomedicines, including the most relevant formulations currently under clinical use.

2. Campthotecin analogues

Since the discovery of CPT and the associated therapeutic limitations, huge scientific efforts are being made to improve the pharmacokinetics, drug resistance, clinical efficacy, and toxicity profiles of the original molecule, by introducing organic ligands [31-36].

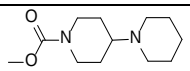
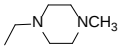
2.1. Structure-activity relationship

In literature, numerous structure-activity relationship (SAR) studies have focused in different modifications of A, B, C, D and E rings of CPT to obtain more active analogs (Table 1 and Fig. 2). It was reported that the complete pentacyclic ring structure was essential for its activity and the planar structure of this system was suggested to be one of the most influential structural element. In addition, the hydroxylactone ring is the most critical region for the activity [35,37-38].

Most derivatives of CPT have been obtained through modifications of the quinolone A-B ring. However, to date, only two of these CPT analogues have been approved for clinical use. Modifications may include substitution at 7, 9, 10 or 11 positions [2, 39-43]. In general, substitution at C12 is unfavorable to biological activity. This loss in the activity can be due to steric and electronic disturbances at the quinoline nitrogen, which might interfere interaction with DNA [44]. Monosubstitution at C9, C10 or C11 by amine or hydroxyl group make it possible to obtain compounds with more antitumoral activity, whereas substitution of positions 9 and 10 by halides or other electron-rich groups and substitution of position 11 with fluorine or cyano groups increase the DNA topo I inhibition ability [44-45]. Moreover, modifications at C10 and C11 positions are adverse for antitumor activity with the exception of the 10,11-methyl or ethylenedioxy, substitutions favorable to DNA topo I inhibition [46-47]. Conversely, other ring combinations in

positions 7 and 9 do not positively affect DNA topo I activity, whereas substitutions at C7 and C10 showed powerful cytotoxicity [48-49].

Table 1. CPT analogues. Most important structural modifications in A, B, C, D, E rings.

Prodrug	Substituent position				IC_{50}[a]	MTD[b]	Clinical	Ref.
	C11	C10	C9	C7	(μM)	(mg/m²/d)		
Irinotecan[c] (CPT-11)	H		H	CH_2CH_3	1.14	290-320	Approved	[30,60]
SN38	H	OH	H	CH_2CH_3	0.09	n/a	-	[61-63]
Topotecan[c]	H	OH		H	0.1	1.5	Approved	[30,36]
Rubitecan (9-NC)	H	H	NO_2	H	0.085	1.5	Phase III	[36,62]
Belotecan (CKD-602)	H	H	H		0.094	0.5	Phase II	[68-69]
Exatecan (DX-8951f)	F	CH_3		—	0.008	0.3-0.5[d]	Phase III	[34,70]
Lurtotecan (GG-211)	—		H		0.006	1.2	Phase II	[34,71]
CPT[c]	H	H	H	H	0.046		-	[36]

[a] *IC_{50}: drug concentration needed to inhibit 50% of the cell growth compared to growth of the untreated control cells. All compounds were tested in a variety of human tumor cell lines.*

[b] *MTD: Maximun tolerated dose*

[c] *Solubility in water at 25 ºC: Irinotecan (HCl)=25 mM; Topotecan (HCl)=100 mM; CPT= 2.9 mM.*

[d] *MTD: 0.3 mg/m²/day for heavily pretreated patients and 0.5 mg/m²/day for minimally pretreated patients.*

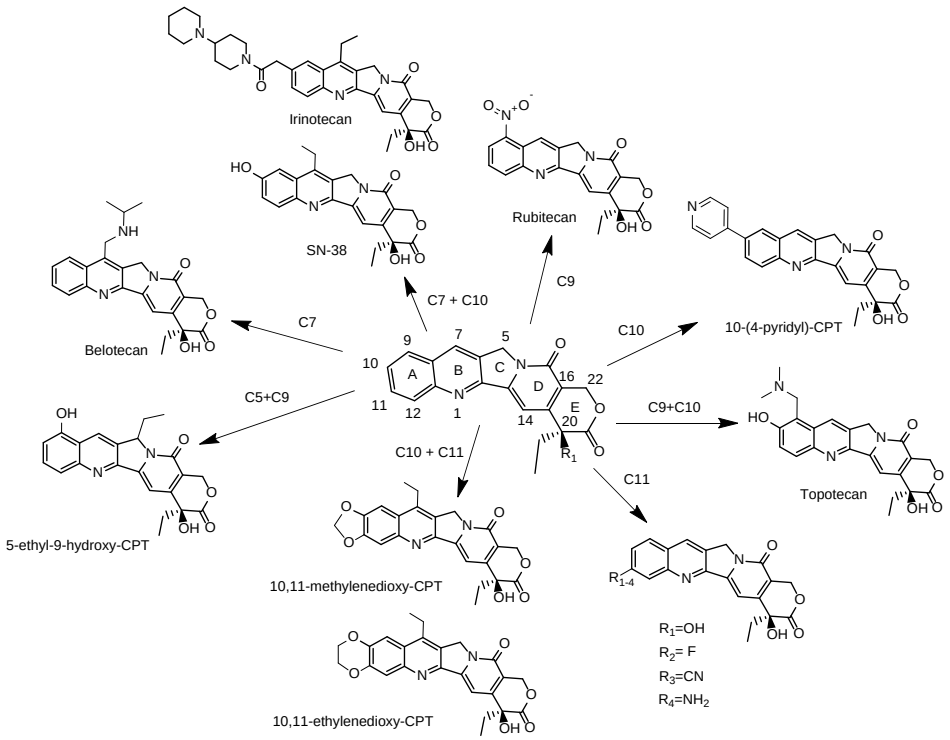


Fig. 2. Scheme of different CPT structural modifications. Where possible, the structure of the resulting analogue is depicted.

Also, a series of 7-substituted CPTs were developed to overcome the instability of lactone ring. All of them showed significantly improved cytotoxic/antitumor potency and stability [50]. On the other hand, few studies have focused on modifications of C and D rings. In general, modifications in position 5, 14 and 17 of these rings reduce cytotoxicity as CPT loses its planarity. Substitution of alkoxy or several other groups at position 5 are reasonably well tolerated when the substituents at position 9 are nitro or hydroxyl groups [51]. Besides, reduction of carbonyl at C17 leads to inactive compounds [34-35,52-54]. At this point, it must be emphasized that E ring and stereochemistry at 20 position are crucial for CPT activity. One of the major drawbacks observed in ring E modification is that, due to the presence of α-OH group, under physiological conditions the lactone ring is cleavaged to inactive carboxylate form. Here, with the aim of increasing lactone ring stability, several derivatives have been reported but with lower cytotoxicity. Also, it is noteworthy that α-hydroxylactone replacement by β-hydroxylactone improves lactone cytotoxicity and stability [35,38].

More recent studies have been focused on different conjugates (ester, amide, carbonate, etc.) at C20 position aimed at new CPT structures. As it will be detailed later on (section 3.1), CPT can be covalently linked by 20-hydroxyl group to numerous organic moieties, including glycerides, poly(amidoamine) and triazine derivatives, polyethylene glycol, poly(N-(2-hydroxypropyl) methacrylamide), polyvinylpyrrolidone, polyethyleneimine, poly(L-glutamic acid), β-cyclodextrins, hyaluronic acid, chitosan and polypeptides. Actually, these systems improve CPT delivery and bioavailability, and may also introduce alternative administration routes to parenteral injection [32,55-58].

2.2. Camptothecin structural derivatives at the clinical stage

Although a variety of CPT derivatives have been developed, only a short list has been investigated in clinical trials [59]. Herein, we try to mention the most representative CPT-

based molecules at the clinical stage. Such illustrative examples are presented in Table 1.

In particular, CPT-11 (also known as irinotecan) was approved by the Food and Drug Administration (FDA) and is used to treat colorectal and other cancers. *In vivo*, CPT-11 is converted into the active metabolite SN38 by the hepatic carboxylesterase enzyme. This metabolite allows to overcome the problems of irinotecan, including intestinal toxicity and neutropenia [60]. Furthermore, SN-38 is one of the most active analogues and shows higher cytotoxic activity than irinotecan [61-62]. However, the principle issue of SN38 is its poor water solubility [63].

Topotecan was the first topoisomerase I inhibitor approved by the FDA and is used to treat ovarian and lung cancers. This compound has shown activity against a broad spectrum of cancers cell lines. A report about Phase II testing [64], mentions that the efficacy of topotecan as second treatment in ovarian cancer was demonstrated among 92 patients. Although this compound was tolerated but different side effects such as alopecia, nausea and vomiting appeared.

Another CPT derivative is rubitecan (9-nitrocamptothecin, 9-NC), a water insoluble analogue currently in Phase III trial. This compound can be metabolically converted *in vivo* to 9-aminocamptothecin (9-AC), which showed anticancer activity in Swiss immunodeficient (nude) mice of the NIH-1 high fertility strain inoculated with different breast carcinoma cell lines (MDA-MD-134 and MDA-MB-231), at a concentration several-fold lower than CPT [65]. Also, rubitecan, showed similar activity than CPT against a broad spectrum of tumor types in human tumor xenograft models [66], and in a Phase II trial over 58 advanced pancreatic carcinoma patients, it achieved >50% tumor shrinkage in 7 cases (16%), being well tolerated by most of patients (only 3 patients discontinued treatment due to toxicity) [67].

Conversely, belotecan (CKD-602) is another derivative of CPT in Phase II that solves the problem of water solubility. This compound showed more activity than topotecan

against a broad spectrum of cancers cell lines and acceptable toxicity [68]. Recently, different clinical studies of belotecan in combination with other chemotherapy drugs such as cisplatin have been investigated [69]. This pharmacological combination demonstrated a promising efficacy in patients with advanced stage small cell lung cancer, although the hematologic toxicity of this regimen requires substantial attention.

Exatecan (DX-8951f) is an intrinsically active compound also in Phase II. This CPT analogue showed higher efficacy against human tumor xenografts (colon, lung, breast, renal) than topotecan and irinotecan. [70]. In an experiment that included over 31 patients with different solid malignancies, the administration of exatecan as a protracted 21-day continuous *i.v.* infusion showed limited systemic toxicity (with a significant reduction of myelosuppresive effects, neutropenia and thrombocytopenia) and efficient anticancer activity.

Finally, lurtotecan (GG-211) is also a water soluble CPT analogue currently in Phase II. This compound showed significant cytotoxicity against HT-29 and SW-48 human colon cancer cells, PC-3 human prostate cancer cell line, MX-1 human breast cancer cells, H460 human lung cancer cell line, SKOV3 human ovarian cancer cells and KB human epidermoid carcinoma cell line [71].

3. Nanoparticles as drug delivery systems for camptothecin

Many types of nanovehicles have been developed for CPT delivery, and these drug delivery systems (DDSs) may be classified in several ways, according to their chemical nature, structure, composition, morphology, etc. Herein, we present a systematic classification of CPT nanocarriers according to their chemical nature in organic, inorganic and hybrid materials, presenting different properties and behaviors [72-75]. These novel nanoplatforms have been designed to solve most of the antitumor drug limitations, improving CPT pharmacokinetics, which is affected by poor water solubility, rapid plasma

clearance, high systemic toxicity and poor selectivity towards cancer cells [36]. A variety of DDSs have been developed and allow CPT incorporation. In addition, these DDSs can conjugate the drug in different ways, as adsorption, encapsulation or covalent bonding [76], working as inert supports to transport the drug to tumor [77]. In this sense, some major advantages of these DDSs over free CPT are: improved solubility, lactone ring stability, half-life extension, biocompatibility and control drug release rates, which allow significant advances at the clinical stage [78-79].

3.1. Organic nanocarriers

The use of organic pharmaceutical nanocarriers to enhance the *in vivo* efficacy of many drugs has been well established in the last two decades [80]. To prepare such smart systems, therapeutic moieties must be simultaneously encapsulated or assembled on the surface [81]. Organic carriers are carbon-based (with the exception of carbon nanotubes that are considered inorganic) and are generally characterized by their high biocompatibility, good solubility or colloidal stability in physiological fluids and quick biodegradability, resulting in excellent pharmacokinetic profiles of the delivered drugs. However, their usual limitations include restricted functionalization (with hinders not only drug loading, but also the incorporation of targeting molecules and cromophores), low stability (especially under enzymatic activity), and the lack of specific physicochemical properties, as optical activity or magnetism, which are useful in diagnostics [82]. In these materials, drugs are often trapped or bound within the matrix.

Although different classifications may be used, we have organized the various organic nanocontainers according to their chemical nature, resulting in five key groups: micelles, liposomes, dendrimers and polymers and biopolymers.

3.1.1. Micelles

Polymeric micelles are expected to increase the accumulation of drugs in tumor tissues by taking advantage of the enhanced permeability retention (EPR) effect [83]. Actually, micelle systems can also incorporate various kinds of drugs into their inner core with relatively high stability by chemical conjugation or physical entrapment [84-85]. Also, the size of micelles can be controlled within the diameter range of 20-100 nm. Therefore, it is expected that the incidence of drug-induced side effects may be decreased due to reduced drug distribution in normal tissues [86].

At this point, polymeric micelles composed of various poly(ethylene glycol)-poly(aspartate ester) block copolymers have been physically loaded with CPT, to improve drug aqueous solubility and stability in biological fluids [87-89]. Here, it was observed that the stability of CPT-loaded micelles depended on the amount of benzyl esters and poly(ethylene glycol) (PEG) length in the polymers. After intravenous administration of CPT-loaded micelles to male ddY mice *via* lateral veins, it was concluded that sample using PEG 5000, 27 aspartic acid (Asp) units and 57-75% benzyl esterification of Asp residue was the most stable formulation, showing an extended circulation period in blood stream [82]. However, it is not clear if CPT is safely packaged in these micelles, which is crucial to avoid premature release before reaching tumor cells.

Another nanomicelle system was developed by using α,β-poly[(N-carboxybutil)-L-aspartamide] (PBAsp) with different CPT loading (PBAsp-CPT), through esterification between PBAsp carboxyl groups and CPT 20-hydroxyl [90-91] (Fig. 3). Subsequently, spherical nanomicelles were produced in aqueous medium. In this case, CPT was sequestered toward the core whereas PBAsp backbone formed the hydrophilic shell. Unfortunately, PBAsp-CPT solubility decreased quickly as the amount of CPT increased. Anyway, *in vitro* cytotoxicity assays of PBAsp-CPT (2,1% CPT) against L929 mouse muscular cell line showed less toxicity than free CPT (5-10% activity drop depending of CPT concentration) due to its linear sustained drug release profile.

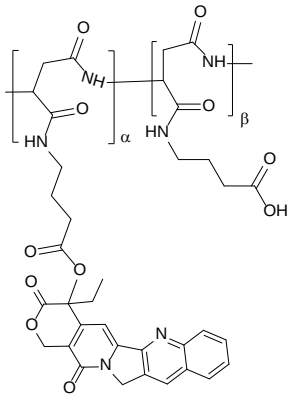


Fig. 3. Schematic illustration of the conjugate made of α,β-poly[(N-carboxybutil)-L-aspartamide] and CPT. (Reprinted from ref. 90, Copyright © 2010, with permission from Elsevier)

In addition, CPT was also encapsulated in different quantities in 45 nm diameter micelles of poly(ethylene glycol)-poly(L-lysine) block copolymer and 3-3'-dithiodipropionic acid. Then, it was studied CPT release in response to cytosol reductive conditions was studied by exploiting the permeabilization of endosomes upon light irradiation (using a halogen lamp with a 400-700 nm band-pass filter) induced by photosensitizer agents, such as Photofrin. Experiments in BALB/c nu/nu mice inoculated subcutaneously with AY27 urothelial cancer cells confirmed that after endosomal permeabilization CPT loaded micelles escaped (from endocytic vesicles) into the cytosol, promoting drug release of more than 70% from micelles in the irradiated tissue (fluence rate to induce the photochemical internalization effect, 100 mW/cm^2; time, 1000 s) and better antitumor activity than the free drug [92].

3.1.2. Liposomes

Liposomes are a well-studied biocompatible carrier playing a key role in nanotechnology-based drug delivery [93]. Liposomes protect the encapsulated drugs form structural transformation or chemical degradation by isolating them from the surrounding environment. However, formulating CPT liposomes is challenging due to different technical issues, as the drug shows poor solubility in nearly all pharmaceutically

acceptable solvents, as well as it associates with simple phospholipid bilayers. Actually, the outcome in most of these approaches is hampered by incomplete separation of free and liposomal CPT, which affects to biological activity and biodistribution. In that sense, a study over several CPT liposome formulations of different nature (cationic, anionic and neutral phospholipids) prepared by drug encapsulation, reported cationic compositions as the best option to increase CPT water solubility [94].

In one of the first attempts to deliver CPT within liposomes, the lipid-complexed formulation (particle size range 20-200 nm) showed significant advantages with regards to the naked drug for intravenous administration in clinically relevant lipid-drug ratios (12.5:1 w/w) [95]. CPT-loaded liposomes (LC-CPT) had an *in vitro* antitumor activity similar to that of free CPT and displayed similar cytotoxicity against multidrug resistance (MDR-1) negative human cervix carcinoma KB-V1 cells and MDR-1 positive human cervix carcinoma KB-3-1 cell line. However, the biodistribution of CPT in ICR Swiss mice was severely affected by lipid complexation, as CPT achieved the greatest concentration in pulmonary parenchyma, while liposomes accumulated mostly at gastrointestinal tract, probably due to the rapid processing of LC-CPT in liver and elimination *via* the gut. Furthermore, LC-CPT showed higher antitumor activity than free CPT in *in vivo* testing over B6D2F1 mice bearing intraperitoneal tumors of L1210 mouse lymphocytic leukemia cells or P388 mouse leukemia cell line.

CPT-loaded liposomes with average particle size of about 150 nm were synthesized by a lipid-film hydration method [96]. In a first step, liposomes were formulated by addition of an artificial lipid with a phenyl group (3,5-bis(dodecyloxy)benzoic acid) to polyethylene glycol-modified liposomes and, subsequently, liposome surface was coated with human serum albumin. Here, CPT was entrapped with about 80% efficiency due to the interaction with phenyl group-containing lipids. After intravenous injection, these protected liposomes showed much higher therapeutic activity than CPT in CDF1 female mice bearing mouse colon adenocarcinoma 26 tumors (tumor volume in CPT-treated

specimens increased up to 5-fold that of liposome-treated mice), with high accumulation in malign tissue (9.6-fold more efficiently than CPT solution).

3.1.3 Dendrimers

Dendrimers have received much attention in drug delivery, as they are highly branched, multivalent and tunable polymers for different architecture, size, shape and surface properties [97]. Dendrimers are usually classified by generation (denominated G), which refers to the number of repeated branching cycles that are performed during its synthesis. Due to their positive charge, cationic dendrimers as poly(amidoamine) (PAMAM) and triazine derivatives conjugate easily with the phenolic 20-hydroxyl group of CPT, resulting in stable nanomedicines. At this point, it has been proved that CPT solubility increases with PAMAM generation [98]. Here, PAMAM dendrimers of four (G4), five (G5) and six (G6) generation were prepared and their solubility in aqueous medium and the interaction with CPT (carboxylate form or lactone form) were evaluated. The study showed that G6 PAMAN enhanced CPT water solubility in comparison with G4 PAMAN. This can be partly explained by the fact that the number of primary and tertiary amines in G6 dendrimer increases and could entrap more hydrophobic molecules inside. However, the cationic nature of dendrimers represents a serious toxicity issue, as it promotes a strong reaction with serum proteins, hemolysis and hepatic toxicity. Furthermore, their chronic toxicity is still to be determined [99].

A relevant PAMAM second generation (G2) model was synthesized with four molecules of CPT per unit and a trigger that allows activation with penicillin-G-amidase (PGA). In this system, the cleavage of the phenylacetamide group by PGA triggered the disassembly of dendron through a known self-immolative reaction sequence (Fig. 4) [100]. This conjugate demonstrated outstanding improvement of CPT cytotoxic activity, as cell-growth inhibition assays indicated that half maximal inhibitory concentration (IC_{50}) value for the dendrimer was between 100- and 1000-fold lower than free CPT against

MOLT-3 lymphoblastic leukemia, JURKAT human leukemia T and HEK-293 human kidney embryonic cell line.

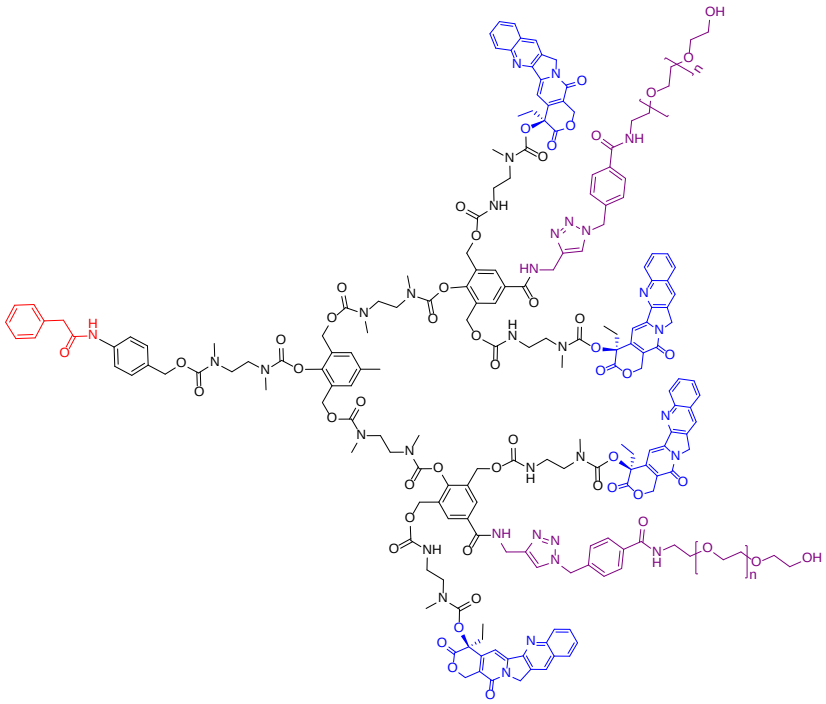


Fig. 4. Molecular structure of second-generation PAMAM with a trigger designed for activation by penicillin-G-amidase. The figure shows CPT in blue, penicillin-G-amidase in red, dendritic structure in black and poly(ethylene glycol)-azide units in purple. (Reprinted with permission from ref. 100. Copyright © 2006 American Chemical Society)

In one important contribution, triazine dendrimer CPT derivatives were synthesized and their cytotoxic activity evaluated against MCF-7 human breast carcinoma cells and HT-29 colon carcinoma cell lines [101]. For this sake, CPT was conjugated with isonipecotic acid through an ester bond, and the obtained secondary amine was highly reactive and combined with the triazine, allowing to obtain the desired dendrimers, which showed almost similar cytotoxicity to free CPT.

Click chemistry has been widely used to improve coupling reaction efficacy in the synthesis of CPT-dendrimer conjugates [102-103]. In a relevant study, CPT was esterified with a spacer 1-azido-3,6,9,12,15-pentaoxaoctadecan-18-oic acid (APO). On the other hand, propargylamine (PPA) and methoxypoly(ethylene glycol) amine were conjugated to PAMAM dendrimer G4.5. Then, CPT-APO was coupled to the modified

dendrimer (G4.5-PPA) *via* click chemistry. Nearly 100% of the CPT molecules were covalently conjugated to the dendrimer and the drug was released by ester bond hydrolysis. This new dendrimer-CPT was more cytotoxic than free CPT (IC_{50} value increase of 185-fold in U1242 human glioma cells).

3.1.4. Organic polymers and biopolymers

In the last years, a myriad of polymer therapeutics, including polymeric drugs, polymer-protein conjugates, polyplexes, polymeric micelles and polymer-drug conjugates [104-105], have received special attention as drug delivery systems and have been established as first-generation nanomedicines. Most of these polymers present advantages such as absence of toxicity, biocompatibility, biodegradability and small particle size, allowing them to circulate in the bloodstream for long periods of time [106-107]. Conversely, the main issues of these materials are their fast biological degradation, which may cause leaching of CPT important quantities of CPT into the blood stream and interstitial fluids, and their variable loading capability due to limited functionalization. Nanoparticles of synthetic polymers, including polyethylene glycol, poly(N-(2-hydroxypropyl) methacrylamide), polyvinylpyrrolidone, polyethyleneimine, poly(L-glutamic acid) and β-cyclodextrins, and natural polymers such as hyaluronic acid, chitosan and polypeptides, are the most commonly reported for CPT delivery [108-109]. In addition, the presence of a covalent chemical bond between a water-soluble polymeric carrier and the bioactive molecule confers enhanced solubility and stability, allowing to improve the pharmacokinetic profile of the bioactive molecule [36, 110-112].

CPT can be encapsulated in hydrophobic polymers or amphiphilic copolymers for safety delivery. At this point, one interesting approach that allows exceptionally high drug loading is to polymerize the drug itself. In one representative example, a dimeric CPT derivative, CPT-SS-CPT, bearing a trigger responsive domain was used as the core-constructing unit of the nanoparticle [113]. Here, CPT molecules are stably conjugated *via* carbonate linkages that are subject to triggered bond cleavage and subsequent drug

release by a reducing reagent (Fig. 5). Upon triggering, cleavage of the disulfide bond in CPT-SS-CPT would result in decomposition of the drug dimer, releasing CPT in its authentic form. Unfortunately, CPT-SS-CPT has much lower water solubility (less than 10 ng/mL) than CPT (3 μg/mL), presumably due to stronger intermolecular interactions between molecules. However, when CPT-SS-CPT was encapsulated in methoxy poly(ethylene glycol)-poly(lactide) (mPEG-PLA, Mw 3500-5000) empty micelles, drug-loaded nanoparticles stable in phosphate buffer saline (PBS) and human serum for at least 3 days were formed, with particle diameter of about 180 nm. The anticancer effect of these CPT-SS-CPT/mPEG-PLA nanoparticles was then evaluated *in vitro* by MTT assay over HeLa human cervix carcinoma cell line. The IC_{50} of CPT-SS-CPT/mPEG-PLA was 114 nM, compared to 17 nM for free CPT, but the main advantages of this nanoconjugate are the lack of systemic toxicity, as CPT release only takes place through disulfide bond reduction by intracellular thiols, and the stabilization of the lactone ring.

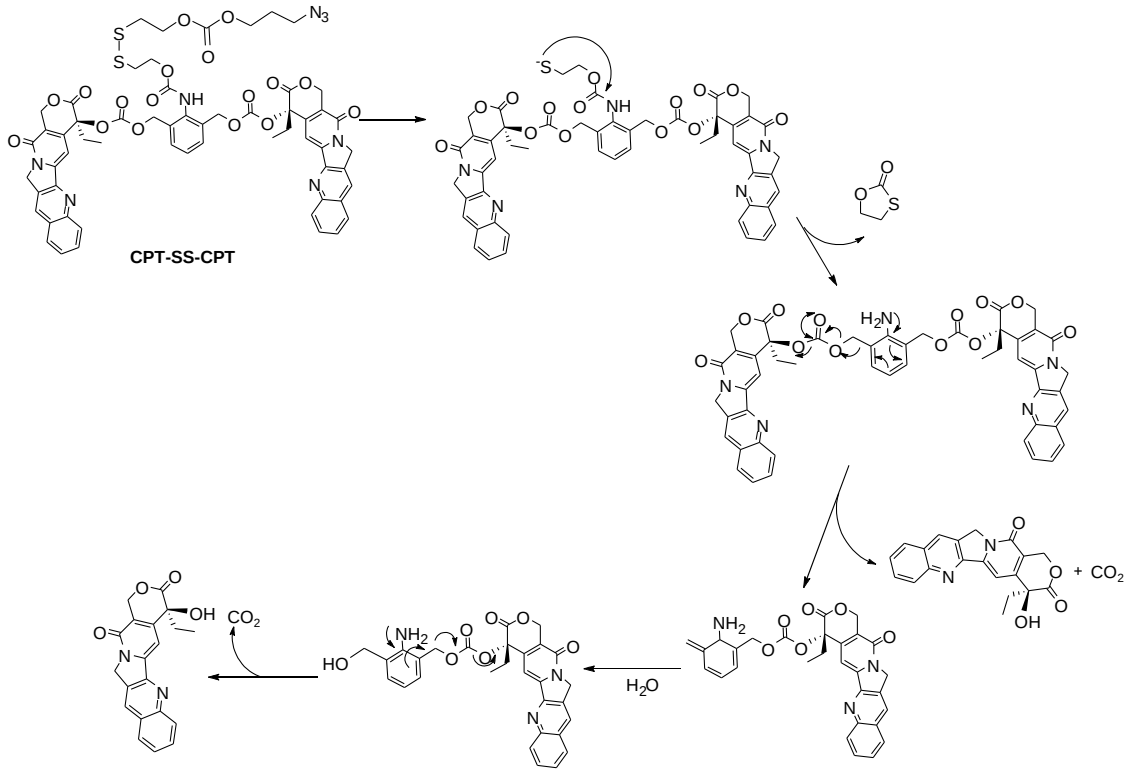


Fig. 5. Chemical structure of dimeric CPT derivative, CPT-SS-CPT and release mechanism of thiol-triggered drug release. (Reprinted partially with permission from ref. 113. Copyright © 2015 American Chemical Society)

One of the best performances to improve CPT stability and solubility in biological conditions has been to covalently conjugate it with a β-cyclodextrin (β-CD) derivative condensed with difunctionalized PEG comonomers, obtaining linear high molecular weight (over 50 kDa) β-cyclodextrin-CPT based polymers [114-117]. For this purpose, a CPT-glycine ester derivative was prepared, which was subsequently linked to the β-CD derivative by amide bond (Fig. 6). This way, the solubility of CPT is increased by more than three orders of magnitude [114]. Moreover, this nanomedicine showed higher concentration in tumor and superior efficacy than irinotecan in the treatment of CD-1 female athymic nude mice bearing LS174T human colon carcinoma, HT-29 human colorectal carcinoma, H1299 human non-small-cell lung cancer model or Panc-1 human pancreatic cancer cell tumors [115-116,118-119]. As a consequence, a commercial product (CRLX-101, formerly IT-101) has been released, currently under clinical trials.

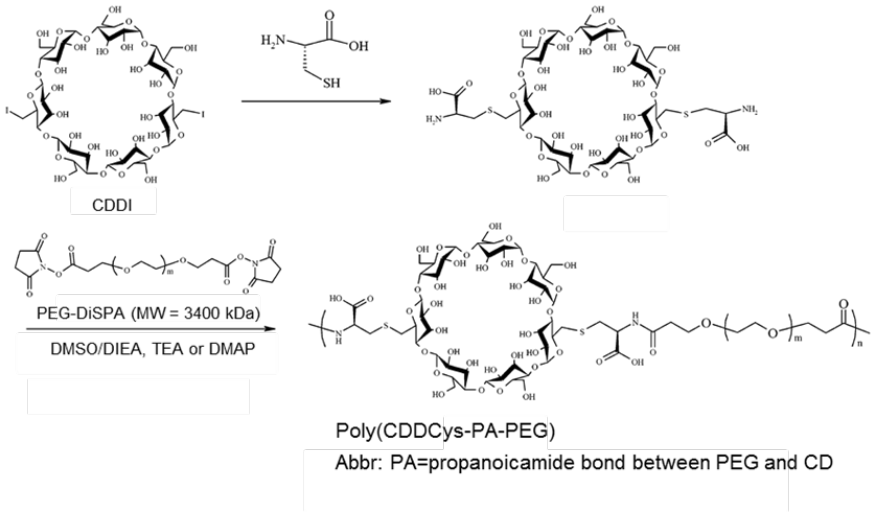


Fig. 6. Schematic illustration of the synthesis steps involved in preparing 6^A,6^D-Diiodo-6^A,6^D-dideoxy-cyclodextrin (CDDI) polymerized derivative. (Reprinted with permission from ref. 114. Copyright © 2003 American Chemical Society)

β-cyclodextrine has also been the aim of further development to improve CPT solubility and stability. In a relevant study, hollow nanospheres were prepared based on β-cyclodextrin-grafted α, β-poly(aspartic acid) (β-CD-graft-PAsp). This conjugate favored the inclusion of CPT and lactone ring stability. *In vitro* cytotoxicity assays in L929 mouse

muscular cell line suggested a decrease of cytotoxicity at higher dose of CPT@β-CD-graft-PAsp [120].

A different CPT delivery system based in β-cyclodextrine is the nanosponge (CDN) prepared to improve CPT transport to prostate cancer cells, including androgen-refractory (DU-145 and PC3 human prostate cancer cell lines) and sensitive (LnCaP human prostate cancer cells) cell lines. CPT was incorporated in the CDN structure and its interaction with the nanosponge was confirmed by thermal analysis. Cell proliferation and clonogenic assays showed a greater responsiveness of CDN-CPT against PC3 and DU145 cell lines, blocking colony formation and cell growth starting at 1 nM dose. Besides, *in vitro assays* against LNCaP cell line, demonstrated that CDN-CPT inhibited androgen receptor gene expression in androgen-resistance prostate cancer patients at 10 nM, whilst CPT was inactive at similar doses [121].

Poly(L-glutamic acid) (PGA) polymers have been combined with CPT by direct esterification [122], or by using a short linker, like glycine [123], obtaining loadings of nearly 40%. The administration of four doses of 7% CPT-loaded PGA by *iv* injection (one single dose every four days, with an equivalent CPT dose of 40 mg/kg) over Sprague-Dawley female athymic nude mice bearing NCI-H322 human lung carcinoma tumors showed a 32-day tumor growth delay with regards to untreated mice, prolonging the median survival of treated mice by 4-fold [122]. Moreover, by increasing polymer molecular weight up to 50 kDa and CPT loading up to 37%, it was reported a tumor growth was delayed to less than half in comparison to untreated mice after a 30 mg/kg CPT equivalent dose over athymic NCr (nu/nu) mice bearing NCI-H460 human lung large cell carcinoma tumors [123].

In the last decade, among different biopolymers, the use of chitosan for drug delivery has been widespread in order to improve biocompatibility and bioavailability of cytotoxic compounds [124]. In one of those applications to prepare a CPT delivery carrier, hydrophobically modified glycol chitosan (HGC) nanoparticles were constructed by

chemical conjugation of hydrophobic 5b-cholanic acid moieties to the hydrophilic glycol chitosan backbone [125]. CPT was easily encapsulated into HGC nanoparticles (CPT-HGC, 280-330 nm in diameter), which showed sustained release for one week. CPT-HGC exhibited significant antitumor effects and high tumor targeting ability towards MDA-MB231 human breast cancer xenografts subcutaneously implanted in nude mice. Tumor growth was significantly inhibited after *iv* injection of CPT-HGC nanoparticles at doses of 10 mg/kg and 30 mg/kg, compared to free CPT at a dose of 30 mg/kg.

More recently, CPT has been conjugated with hyaluronic acid (HA-CPT) with variable molecular weight, in order to overcome the low solubility and poor targeting ability of the naked drug [32]. Here, CPT was incorporated using two linkage molecules such as succinate and adipic acid dihydrazide. The authors studied the influence of the polymer molecular weight over solubility, drug loading and stability. Solubility assays confirmed that these conjugates increase CPT solubility, mostly those samples with lower molecular weight. Furthermore, *in vitro* efficacy assays demonstrated that the antitumor activity of HA-CPT polymers were higher than the naked drug against HepG2 human liver cancer cell line due to an HA receptor overexpression.

A singular and very efficient approach for CPT delivery has been achieved by encapsulating the drug during self-assembling peptide amphiphile (PA) nanofibers through a solvent evaporation technique [126]. *In vitro* efficacy assays against BT-474 human breast cancer cells, MCF-7 human breast adenocarcinoma cell line and SKBR-3 human breast cancer cell line, demonstrated that the intracellular release of the cargo induced cell death with half maximal inhibitory concentration (IC_{50}) values similar to those observed with the naked drug. Furthermore, *in vivo* assays of the PA-encapsulated CPT in a mouse orthotopic xenograft model (Sprague-Dawley female athymic nude mice) of BT-474 human breast carcinoma suggested that the nanomaterial delayed subcutaneous tumor growth.

3.2. Inorganic nanocarriers

Currently, a wide variety of inorganic nanoparticles may have biological applications. They usually show high stability in the biological environment, ease of functionalization (allowing multifunctionality) and unique physico-chemical properties (optical, magnetic and electronic) that can be tailored by controlling particle structure, composition, size and shape. Materials as semiconductor quantum dots, magnetic nanoparticles and plasmonic nanoparticles, among others, have been proposed for imaging and therapy [127,128]. However, despite the interest raised by inorganic nanoparticles as DDSs, most of these systems suffer of biocompatibility issues, mostly related to the toxicity of some structural components and the strong interaction with serum proteins, which results in rapid blood clearance and hepatic removal [129]. Moreover, occasionally, the elimination from organic tissues may be an issue, involving the accumulation in the body of highly toxic elements. Herein, we present a few cases of simple inorganic nanoparticles that have found application for delivery of CPT, metal oxides and carbon nanotubes.

3.2.1. Metal oxide nanoparticles

The most significant use of transition metal oxides for biological purposes are magnetic processes, such as magnetic resonance imaging (MRI) and magnetic induction hyperthermia. Typically, paramagnetic oxide nanoparticles show a critical size of around 10-20 nm, which is dependent on the material, have a large magnetic moment and are stable in physiological fluids [129].

Iron oxide (Fe_3O_4) hollow monodispersed spheres of about 200 nm mean diameter, and composed of small primary nanoparticles with a 30-40 nm average size, were synthesized using a one-pot solvothermal method, and further loaded with CPT by incubation in a chloroform solution [130]. These nanospheres showed negligible drug leakage before cell internalization, and exhibited slightly higher cytotoxicity than free CPT against 786-O, HepG2 and HeLa cell lines, due to the enhanced solubility of the

transported drug. Furthermore, non-loaded Fe_3O_4 hollow spheres showed nearly no cytotoxicity towards the cells, although there is no report on the stability of these nanocarriers in *in vivo* conditions.

Very high CPT loading has been achieved into iron oxide (Fe_3O_4) superparamagnetic nanoparticles (SPIONs) with 14 nm average size obtained through an iron co-precipitation method under alkaline conditions [131]. Formulations containing 13 wt% CPT were produced by incorporation of the drug to a SPION aqueous solution; this increased up to 26 wt% CPT by functionalizing SPION surface with a PEG. The high loading capacity was attributed to the amphiphilic nature of the PEG molecule. Nevertheless, this did not bring about any improvements in cytotoxic activity, as CPT-loaded nanocarriers demonstrated about 10-15% lower apoptotic levels over H460 cell culture than the free drug. This was justified on the basis of CPT slow release profile from the SPION formulations.

3.1.2. Carbon nanotubes

Carbon nanotubes (CNTs) have recently emerged as a new alternative vehicle for transporting and releasing therapeutic molecules, due to their high surface areas [127,132]. However, several concerns relating to pharmacokinetics, biodistribution and toxicity (mostly by liver and kidney accumulation), must be solved before they can go into clinical application. In this sense, surface chemical modification becomes a crucial step for preparation of soluble CNTs and efficient drug incorporation [127].

In an attempt to raise CPT water solubility and antitumor efficacy, non-covalent supramolecular attachment on multiwall carbon nanotubes (MWCNTs) has been carried out. Surface oxidation of MWCNTs followed by functionalization with poly(vinyl) alcohol (PVA) allowed CPT loading *via* π-π interactions (Fig. 7) [133]. This construct was found to be very stable in simulated body fluid and, in *in vitro* testing, it was approximately 15-fold more cytotoxic than free CPT against MDA-MB231 human breast cancer and metastatic skin A-5RT3 cell lines.

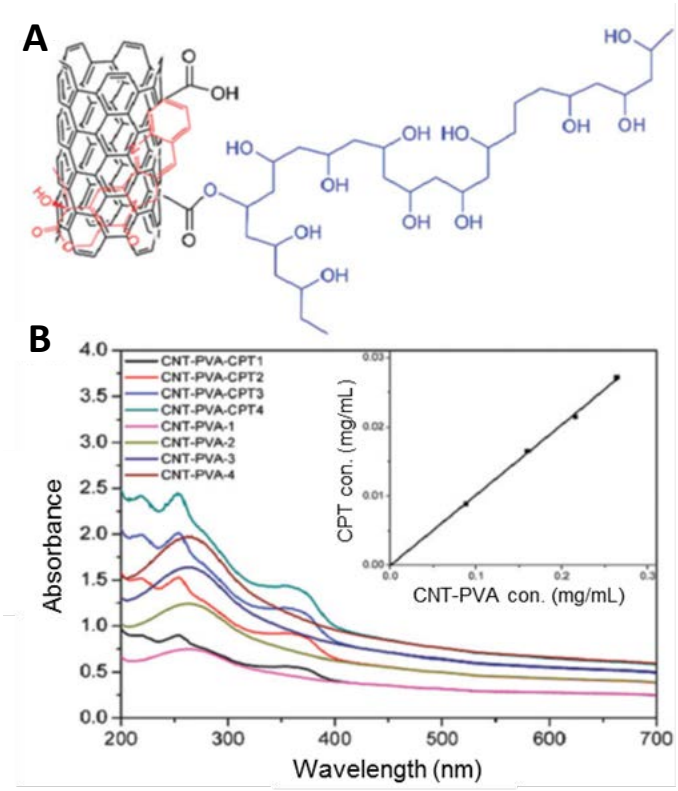


Fig. 7. (A) Schematic depiction of CPT loaded MWCNT-PVA. The figure shows CPT molecule in red, MWCNT structure in black and polymer PVA in blue. (B) UV spectra of multiwall CNT-PVA and CNT-PVA-CPT samples with different concentrations. (Reprinted from ref. 133 with permission of The Royal Society of Chemistry)

Another singular CNT-based material suitable for CPT delivery are glyconanosomes (GNS). These are water-soluble nanodisks created by supramolecular self-organization and photopolymerization of diacetylenic-based glycolipids on single wall carbon nanotubes (SWCNTs) surface, and subsequently used as molecular scaffolds that determine their shape and topology (Fig. 8) [134]. Experiments against MCF-7 human breast cancer cells suggested that CPT-loaded GNS-SWCNTs presented higher antitumor activity than the naked drug (killed more than 65% of MCF-7 human breast cancer cells while CPT alone affected less than 55% MCF-7 cells), which could be explained because the interaction of GNS/CPT could interfere with cellular CPT detoxification mechanism in such a way that intracellular CPT retention time increases, prolonging the span and release time of the drug.

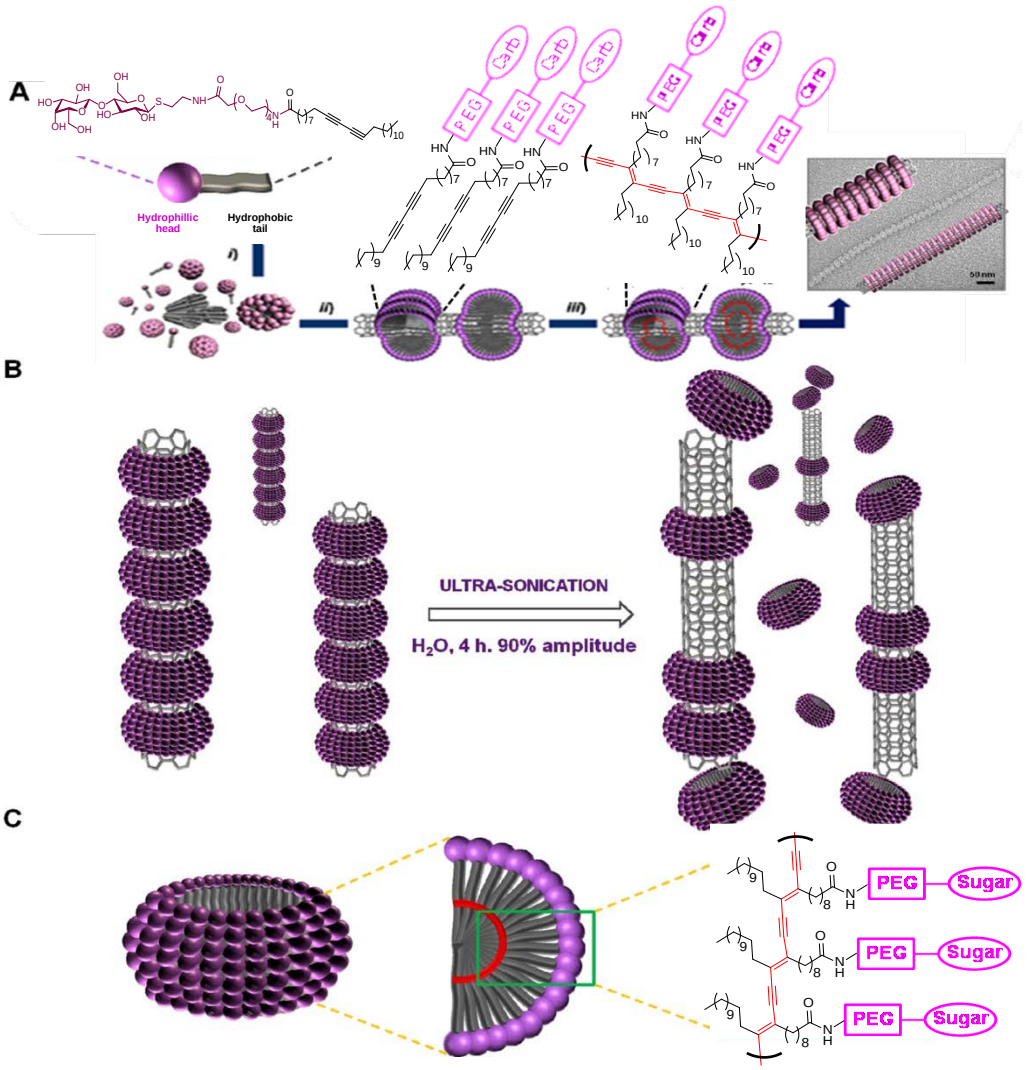


Fig. 8. (A) Schematic representations of the self-organization and synthesis of glyconanosomes (GNS). The inset is a TEM micrograph of SWCNT nanoassemblies and their idealized representative figures. (B) Schematic representation of the ultrasonication setup for obtaining GNS. (C) Structural and chemical composition of a GNS. (Reprinted with permission from ref. 134. Copyright © 2013 American Chemical Society)

3.3. Hybrid nanocarriers

As commented before, fully inorganic systems found strong limitations for biological use, due to possible compositional toxicity and severe interaction with serum proteins. For this reason, inorganic nanocarriers are better handled surface modified or combined with organic components, which provide good stability in physiological fluids, low toxicity and immunogenicity, and minimized reactions with serum proteins, to control their fate in biological environment [129]. These composites consisting of two or more constituents

distributed in the nanoscale or molecular range are hybrid materials. Typically, the hybrid nanocontainers are made of inorganic and organic building blocks, presenting unique properties depending on the spatial and size distribution of their components [135-136]. Actually, efforts have been done to design hybrid devices combining different functions or strengths of the individual components in a synergic way, or even to compensate for the weaknesses of one another [137]. Whilst organic component provides good biocompatibility and better solubility in physiological fluids, the inorganic moiety offers almost unlimited external surface area to bind different functionalities, improving the capabilities of the DDS. On the other hand, the main handicap of these systems is the lack of long-term toxicity studies about the possible interactions within the body of the different components.

A myriad of compositions and models have been reported with potential use in nanomedicine. According to their structural configuration, we have distinguished three different types of hybrid materials with applications as DDS: organic-inorganic nanoparticles, core-shell systems and metal-organic frameworks. However, in this section we only present a brief description of some of the most representative examples of the different systems proposed, as most of these hybrid nanocarriers for CPT delivery carry molecular devices sensitive to specific physico-chemical stimuli, which act as triggers for selective release activation at the targeting tissues and cells. These last are described in detail in the next section.

3.2.1. Organic-inorganic nanoparticles

Organic-inorganic hybrid materials combining the individual advantages of both components while overcoming their intrinsic drawbacks have received extraordinary attention in the last decade. In the case of biomedical applications, their complex chemical composition, their variable structure, but mostly the inherent multifunctional property, offer plenty of room for the development of novel systems that are able to carry out associate actions inside the body, as imaging and therapeutics.

Mesoporous silica nanoparticles (MSNs) modified with organic ligands are probably the most widely used inorganic material for hybrid DDS development [138]. These particles have large surface areas and porous interiors that can be used as reservoirs for storing hydrophobic drugs as CPT. Moreover, particle surface and pore size can be easily tailored to selectively store and deliver different molecules. In this context, MSNs were stabilized in aqueous medium by surface modification with methylphophonate groups, which reduced aggregation [139]. Then CPT was absorbed within the 2 nm diameter pores and the particles were incubated with different cells lines (PANC-1 human pancreatic carcinoma cell line, Capan-1 human pancreatic ductal adenocarcinoma cell line, AsPc-1 human pancreatic adenocarcinoma cells, SW 480 human colon adenocarcinoma cell line and MKN 45 human gastric cancer cell line) to determine if they were able to transport the hydrophobic drug into the cancer cell. In all cases, results indicated that the cytotoxic efficacy of CPT-loaded MSNs was very similar to that of the dissolved CPT. In an ulterior study, folic acid was incorporated to the surface of MSNs (FA-MSNs) as targeting molecule to pancreatic cancer cells. Then, *in vivo* biodistribution, therapeutic efficacy and biocompatibility of the CPT-loaded FA-MSN were evaluated in BALB/cAnNCrj-nu nude mice bearing MCF-7 human breast carcinoma tumors. These authors showed that nanoparticles were able to repress subcutaneous tumor growth with a minimum therapeutic dose of about 0.5 mg/kg CPT-loaded FA-MSN for complete tumor growth inhibition. Inhibition of tumor growth was also complete in the CPT-treated group but with clear signs of toxicity in blood analysis, as high concentration of liver enzymes, hypoalbuminemia and hypoproteinemia, elevated creatinine kinase and decreased levels of calcium and phosphorous. Moreover, one mouse in the group treated with CPT was euthanized on the 51st day due to manifestation of severe toxicity. Conversely, the group treated with CPT-loaded FA-MSN only presented mildly elevated blood concentration of liver enzymes [140-141].

In another MSN-based model, MSNs were functionalized with hyaluronic acid (MSN-HA) to overcome agglomeration issues. The cellular experiments showed that MSNs-HA is capable of selectively targeting specific cancer cells over-expressing the CD44 protein, leading to rapid and concentration dependent uptake by the cancer cells through the receptor-mediated endocytosis mechanism [142]. Subsequently, CPT was encapsulated in the pores, and the system showed enhanced cytotoxicity to HeLa cells compared to both free CPT and CPT-loaded MSNs-HA in the presence of excess free HA.

The integration of functional organic fragments into the framework of mesoporous silica to produce periodic mesoporous organosilica materials (PMOs) was reported in 1999 by three different groups [143-145], by using bridged organoalkoxysilane precursors $(R'O)_3Si$-***R***-$Si(OR')_3$ (***R***: organic group). PMOs feature molecularly organic-inorganic hybrid compositions where the organic ***R*** moiety homogeneously distributes within the whole framework without blocking pore channels. The recent development shift from bulk materials to nanosized PMOs has resulted in mesoporous organosilica nanoparticles (MONs) with biomedical applications [146]. As a consequence of this hybridization strategy the chemical nature of the silica framework can be significantly altered, offering a number of specific biological effects and functions suitable for biomedicine, as tunable biodegradation and improved biocompatibility. However, with regards to MSNs, the *in vivo* evaluation of the biological effects and the biosafety of MONs is still in its infancy, and complete studies of biodistribution, excretion, biodegradation, and toxicity are still pending [147]. In this context, in a singular study, monodisperse MONs co-doped with fluorescence resonance energy transfer (FRET) cascades composed of fluorophores anthracene (Anth), naphthalimide (Naph) and biphenyl (Biph) at various ratios were prepared by a modified Stöber method, and CPT was loaded inside the pores [148]. Next, release experiments in PBS demonstrated that sample containing the three fluorophores (Biph/Anth/Naph) presented quicker release profile than samples with two fluorophores (Biph/Anth) or with only Biph. Actually, this CPT tunable retention ability is

governed by the quantity of incorporated fluorophores and their polarities, which follows the order of Naph > Anth ~ Biph. Moreover, although the release rate can be more finely tuned in an asynchronous way (a single drug) by varying the ratio between fluorophores, it also possible monitoring the synchronous discharge of several drugs. In this sense, it can be said that drug releasing performance of MONs can be well correlated with its color. Studies on the cellular uptake efficiency were carried out on HeLa human cervix carcinoma cell line, resulting that by varying the ratio between the different fluorophores, a colorful bioimaging system benefited from FRET should be obtained. Also, the cell viability evaluated using MTT assay indicated similar growth inhibition potential for MONs than the free drug.

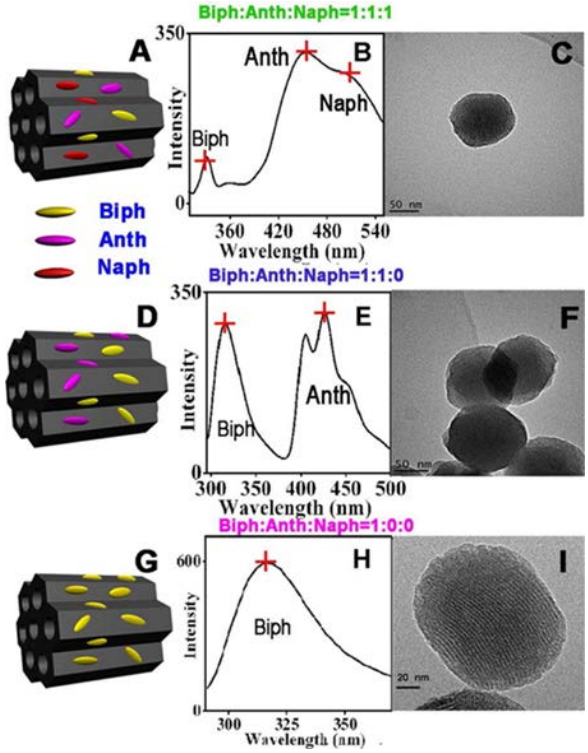


Fig. 9. Illustrations (A, D, and G), emission spectra (B, E and H) and TEM images (C, F and I) of monodisperse mesoporous organosilica nanoparticles co-doped with FRET cascades composed of triple fluorophores: anthracene (Anth), naphtalimide (Naph) and biphenyl (Biph). The red plus sings indicate the maximum absorption wavelength of different fluorophores. (Reprinted from ref. 148. Copyright © 2012 American Chemical Society)

Closely related to MSNs, mesoporous bioactive glass nanoparticles, with higher specific surface area and pore volume than conventional bioactive glasses, have been recently

proposed for hybrid DDS development [149]. In a significant study, the mesoporous bioactive glass SiO_2−CaO−P_2O_5,(MBG-OH) was functionalized with folic acid and fluorescein isothiocyanate silanes (MBG-FA) and, subsequently, CPT was absorbed within the pores (MBG-CPT) [57]. *In vitro* testing over HeLa human cervix carcinoma cells, which are overexpressed folate receptors, and L929 murine fibroblast cells, which are deficient folate receptors, suggested that while loading efficiency was better for FA free nanoparticles, MBG-CPT showed higher cytotoxicity in HeLa cell line than MBG-OH because of the increased cell uptake of anticancer drug delivery vehicles mediated by the FA receptor. Confocal microscopy experiments confirmed that nanoparticles enter the cells by endocytosis and that cell uptake was mediated by the folate receptor.

Conversely, stable hybrid DDSs can also be obtained by modification of an organic matrix with inorganic components. In an illustrative example, silane-modified amphiphilic chitosan was synthesized by anchoring a coupling agent, (3-aminopropyl)triethoxysilane, to an amphiphilic carboxymethyl-hexanoyl chitosan. Further self-assembly in a CPT solution led to drug loaded nanovesicles with stable polygonal geometry, consisting of ordered silane layers of 6 nm in thickness [150]. The hybrid presented well-controlled encapsulation and release profile for CPT, which are strongly conditioned by chemical composition and silane concentration. These nanovesicles exhibited excellent biocompatibility and cellular internalization capability in ARPE-19 human retinal pigmented epithelium cell line, confirming by confocal microscopy experiments that nanomaterial entered the cytosolic compartment to release the cargo. Unfortunately, no cytotoxicity study comparing with the naked drug was provided.

In another organic-inorganic model CPT was first incorporated into micelles derived from negatively charged biocompatible surfactants, such as sodium cholate, and these negatively charged micelles were then encapsulated in nanoparticles of magnesium–aluminum layered double hydroxides (LDHs) by an ion exchange process [151]. The encapsulation method allowed for an approximately 3-fold increase in CPT solubility.

When administered to 9L Glioma cells, the nanobiohybrid containing CPT resulted in significantly lower survival times compared to untreated cells, or to cells incubated with the surfactant, the pristine LDH, or water (delivery medium). In addition, LDH surface modification with bifunctional succinimidyl compounds allows the attaching of active targeting biomolecules, which may direct these nanohybrids towards specific cells or subcellular components, increasing their cell uptake ratio. Although these nanocomplexes showed slightly lower cytotoxic activity than free CPT, they look like a promising biocompatible model for CPT delivery, allowing drug administration in a dose-controlled fashion due to the good dispersion of the complexes in water.

3.2.2. Core-shell systems

Complex nanoscopic core–shell architectures are potentially useful for biomedical applications, for example, as image developers, smart sensor materials or as DDSs [152]. In this context, the target of designing multifunctional nanoparticle architectures for biomedical applications has strongly stimulated progress in synthesis of core–shell nanoparticles with increasing structural complexity. The incorporated functionalities may provide high resolution imaging and sensing (e.g., using fluorescent molecules, semiconductor nanocrystals and/or metal nanoparticles), accurate local temperature manipulation in cancer hyperthermia (magnetic nanoparticles, plasmonic nanoparticles), and controlled drug delivery and release, combining organic and supramolecular chemistry with colloidal nanoscience. Here, different models for delivery and controlled release of CPT have been reported, the most significant of which are described below.

Core-shell organic nanocapsules for oral delivery of camptothecin were formulated with a core made of amphiphilic cyclodextrins (CDs) and an external coating of chitosan (CS) [153]. These formulations were in the range of 180-220 nm, with narrow size distribution. CPT was loaded in the inner polymer and then positively charged chitosan was added to cover the particles. The resulting nanocapsules were found to be stable in simulated

gastrointestinal media and proved favoring CPT permeability through an artificial mucus gel layer and through Caco-2 (human colorectal adenocarcinoma cells) membrane. *In vivo* animal studies in CD1 female mice demonstrated that CPT-loaded CD@CS nanocapsules promoted drug absorption at the intestinal tract *vs* stomach uptake. This could be an interesting vehicle for CPT oral administration, although it does not solve the important issue of systemic toxicity.

One of the most studied core-shell systems is that obtained by protecting a metal nanoparticle with an organic coating. In one representative example, multifunctional nanocarriers of gold nanoclusters as core and folate (FA)-conjugated amphiphilic hyperbranched block copolymer (based on poly(L-lactide) inner arm and FA-conjugated sulfated polysaccharide outer arm) as shell, were synthesized for CPT targeted delivery (Fig. 10) [154]. These nanocarriers presented a release pattern curve at different pH in two stages, and an initial rapid release (1 h) was followed by a sustained release period

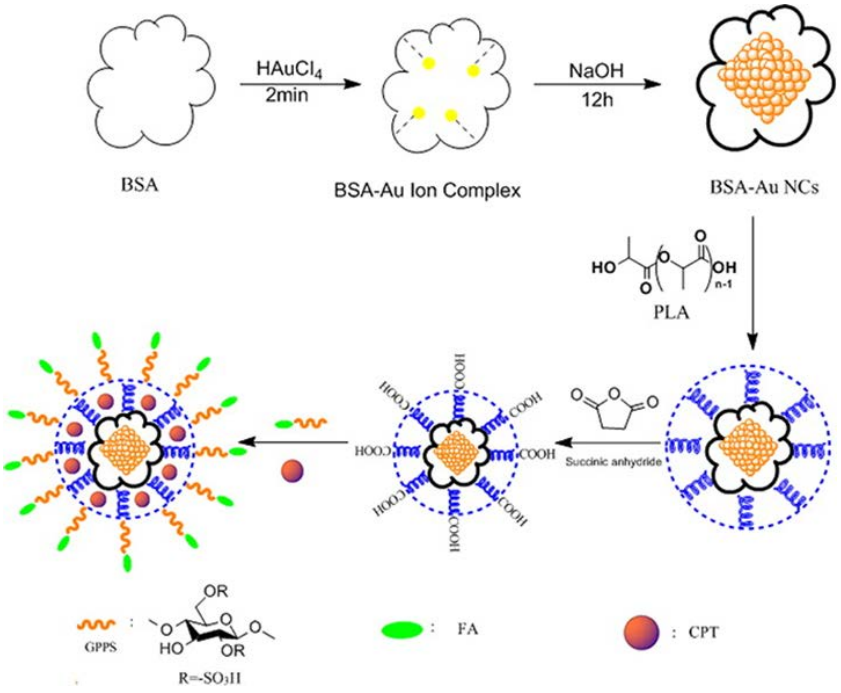


Fig. 10. Schematic illustration of gold nanoclusters as core and folate (FA)-conjugated amphiphilic hyperbranched block copolymer as shell. (Reprinted with permission from ref. 154. Copyright © 2012 American Chemical Society)

(up to 15 h), and then reached a plateau. Cytotoxicity studies over HeLa cells showed that, with regards to the free drug, CPT-loaded nanocarrier provided slightly higher (about 10%) anticancer activity, also gaining specificity to cancer cells with overexpressed FA receptor moieties.

Fully inorganic core-shell hybrids can also be used for CPT delivery. In a most usual configuration, the nanocarrier is obtained by coating a metal nanoparticle with a silica outer layer, CPT being encapsulated inside the silica pores. In this sense, recently, our group carried out combined chemo and photothermal therapy in *in vitro* testing by means of multifunctional nanoparticles formed by plasmonic gold nanoclusters (GNCs) with a protecting shell of porous silica that contains CPT (GNC@SiO_2) [155]. This therapeuticnanoplatform associated the optical activity of small GNCs (longitudinal surface plasmonresonance peak, LSPR, at 660 nm) to the cytotoxic activity of CPT simultaneously released for the efficient destruction of cancer cells. A method was used for the controlled assembly of 15 nm diameter gold colloids into stable clusters with a tailored absorption cross-section in the vis/NIR (near-infrared spectroscopy) spectrum (Fig. 11). Clusters were further encapsulated in an ordered homogeneous mesoporous silica coating that incorporates CPT molecules within the pores. After internalization in 42-MG-BA human glioma cells, GNC@SiO_2 were able to produce effective photothermolysis under femtosecond pulse laser irradiation of 790 nm. Moreover, the simultaneous release of CPT during the process increased cell death up to 70%. On the other hand, control experiments with no irradiation reported 45% cell death for CPT-loaded GNC@SiO_2 nanoparticles and 30% cell death for the free drug. This therapeutic model could be of interest for application in the treatment and suppression of non-solid tumors like glioblastoma multiforme.

In a similar way, mesoporous silica-coated gold nanorods (GNR@SiO_2) can find applications in cancer therapy due to their optical properties (LSPR at 770 nm) and cytotoxic activity (CPT loading in the mesopores). Moreover, tLyP-1, a kind of tumor

homing and penetrating peptide, was engrafted to GNR@SiO_2 [156]. The obtained GNR@SiO_2−tLyP-1 was loaded with CPT and tested over hMSC human mesenchymal stem cells, HeLa human cervix carcinoma and MCF-7 human breast cancer cell lines. After cell internalization, core-shell nanorods were irradiated by NIR illumination. Then, all cells were killed instantaneously by the increase in temperature caused by GNR@SiO_2−tLyP-1 surface plasma resonance and CPT cytotoxic activity. Moreover, the systematic toxicity of CPT on human mesenchymal stem cells was minimized, because the GNR@SiO_2−tLyP-1 selectively targeted and penetrated into the tumor cells, and little hydrophobic CPT was released into the culture medium or blood.

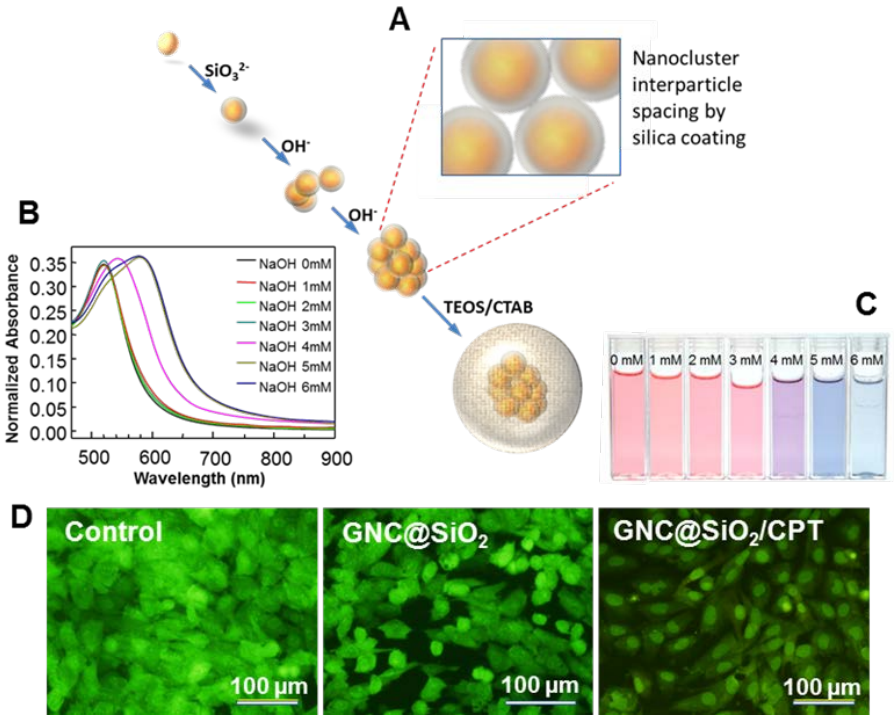


Fig. 11. (A) Schematic of synthesis and controlled aggregation of gold colloids protected with a thin silica layer into gold nanoclusters (GNC@SiO_2). (B) UV-vis/NIR absorbance spectra of gold nanoclusters obtained at different NaOH concentration. (C) Photograph of the different colloidal gold dispersions. (D) Fluorescent microscope images of 42-MG-BA human glioma cells incubated with GNC@SiO_2 (with and without CPT) after laser irradiation. A control with no nanoparticles (NP) is also shown. (Adapted from ref. 155 with permission of The Royal Society of Chemistry)

3.2.3. Metal organic framework nanoparticles

Due to their porous ordered structure and virtually infinite combinations of metals and ligands, metal-organic frameworks (MOFs) can exhibit exceptionally high surface areas with large pore sizes. Consequently, they have been recently proposed for applications in loading and controlled release of several drug molecules [157]. However, MOFs need to be scaled down to the nanoregime to form nanoscale metal-organic frameworks (NMOFs) to be used as delivery vehicles for imaging agents and drug molecules [158]. NMOFs possess some potential advantages over existing nanocarriers given their compositional and structural diversity, which allows for the synthesis of different shapes, sizes and chemical properties, and their biodegradability, a result of relatively labile metal-ligand bonds. Surprisingly, despite the huge potential of these nanomaterials for novel DDSs development, stability in physiological fluids determination and identification of potential hazards associated to their components are still a matter to investigate [159]. Very few toxicological studies concern NMOFs, usually limited to specific cell lines, which makes it difficult to compare the results obtained. In this context, there are very few examples of MOF application to CPT delivery yet, most of them focused on the structure of ZIF-8 [160,161].

A general synthetic route was optimized to encapsulate several small molecules, including fluorescein and CPT in monodisperse zeolitic imidazolate framework-8 (ZIF-8) uniform of 70 nm particles with single-crystalline structure (Fig. 12) [160]. Evaluation of fluorescein-encapsulated ZIF-8 nanospheres in MCF-7 human breast adenocarcinoma cell line demonstrated cell internalization and minimal cytotoxicity. The 70 nm particle size facilitates cellular uptake, whereas ZIF-8 framework easy dissociation results in quick endosomal release of the small-molecule cargo. Here, it was shown that encapsulated CPT ZIF-8 particles show enhanced cell death, which involves cell uptake and intracellular release of the drug.

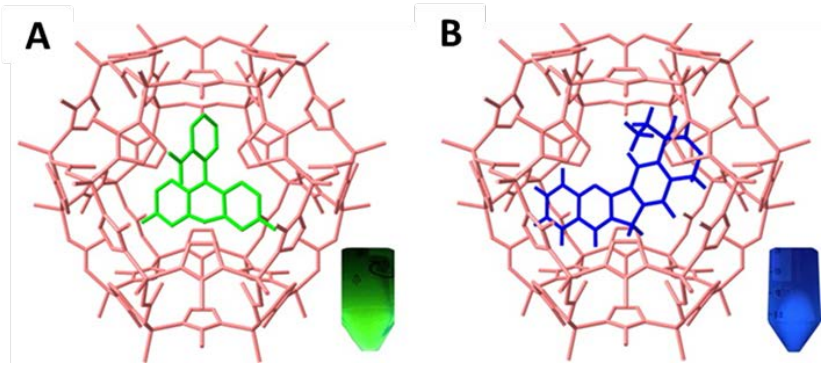


Fig. 12. Schematic illustration of MOF application for small molecule delivery (fluorescein in green (A) and CPT in blue (B)) inside of ZIF-8. CPT molecule fits well within the microporous framework, which loads a maximum of 2 % drug. (Reprinted from ref. 160. Copyright © 2014 American Chemical Society)

Overall, the incorporation of CPT to a nanocarrier definitively improves its therapeutic efficacy. All vehicles provide a clear increase of drug stability, as the encapsulated or bonded molecule is protected from enzymatic degradation and lactone ring cleavage during its transit through blood stream and tissues to tumor location. Moreover, colloidal stability of these formulations can boost drug solubility several orders (e.g., β-cyclodextrins), which gathered to the stability rise improves the bioavailability. However, the most significant effect of incorporating CPT into a nanocontainer is the dramatic toxicity reduction and therapeutic window extension, as the transported molecule has very little activity before it is discharged at the tumor place. As a consequence, the *in vivo* antitumor activity increases with regards to the free drug, due to an improved bioavailability and the possibility to increase the dose with no side effects. In addition, many of these nanomedicines offer the possibility of delivering several drugs in a row, which is very important for the treatment of MDR cancer, and allow for the incorporation of targeting molecules that favor selective therapy. Finally, inorganic and hybrid materials can present unique properties (e.g., optical activity, magnetism, semiconductor activity), that enhance the potential of these systems in therapeutics and diagnostic.

4. Stimuli responsive systems

These are currently the most representative DDSs in Nanomedicine. The main characteristic is their ability to deliver a therapeutic agent into the target cells with no previous release. This is due to the accurate control of the release process through a specific intracellular stimulus, which minimizes undesired side effects. Stimuli-responsive nanocarriers can be classified in endogenous or exogenous, depending on the stimulus which activates the drug release mechanism. Examples of endogenous stimuli are pH, redox potential and enzyme activity. On the other hand, classical exogenous stimuli include magnetic fields, ultrasounds, light and temperature [162-165].

4.1. pH-sensitive systems

Several pH-sensitive DDSs have been developed based on the pH difference between normal and tumor tissue. Here, some of these platforms take advantage of the fact that, in some cases, tumor tissue pH profile is slightly lower than normal tissue. However, the heterogenicity of extracellular pH both within and between tumors poses a significant challenge, as low extracellular pH regions are likely to be less accessible to nanoparticles that do not distribute readily within the tumour interstitium. This factor, together with the lower pH values inside endosomes, suggests that enhancing endosomal escape is the most realistic objective for pH-sensitive nanocarriers in the short term [166].

Polymeric, pH-responsive micelles have been prepared by a solvent evaporation method. For this purpose, hydrophilic methyl ether poly(ethylene glycol) (MPEG) and biodegradable poly(β-amino ester) (PAE) were copolymerized to give MPEG-PAE block copolymer (Fig. 13). Then, CPT was encapsulated into the micelles (CPT/MPEG-PAE) through hydrophobic interactions between drug and PAE. At pH 6.4-6.8 CPT/MPEG-PAE were demicellized and the encapsulated CPT was rapidly released from these nanocarriers, although under pH 7.4 the release rate was clearly slower. *In vitro* cytotoxicity assays in MDA-MB231 human breast tumor cells confirmed that these

micelles provoked severe cytotoxicity within an acidic environment due to rapid CPT release, whereas cytotoxicity decreased at physiological pH [167].

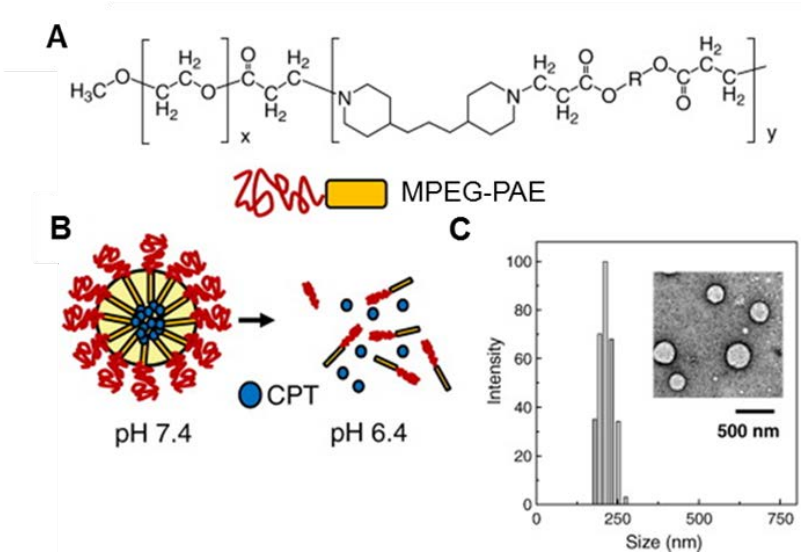


Fig. 13. (A) Chemical structure of methyl ether poly (ethylene glycol) - poly(β-aminoester) block copolymer (MPEG-PAE). Letters x and y indicate the number of times that the block polymer is repeated. (B) Schematic diagram of pH-responsive CPT/MPEG-PAE micelles at weakly pH condition. The figure shows CPT in blue, and in red and yellow the MPEG-PAE block copolymer. (C) Size distribution of MPEG-PAE micelles in PBS at pH 7.4 by dynamic light scattering (inset: TEM image). (Reprinted from ref. 167, Copyright © 2010, with permission from Elsevier)

Another pH-sensitive nanoscaled system for CPT delivery has been obtained by conjugation to a biocompatible, hydrophilic copolymer of mucic acid and polyethylene glycol (MAP). When this polymer-drug conjugate was placed in water, it self-assembled into MAP−CPT nanoparticles of ca. 30 nm [168]. Then, antibody herceptin was complexed with MAP-CPT to form a targeted nanoparticle. A strong dependence of release rate with pH was observed but, unlike the standard behavior, as pH increased from 6.5 to 7.4, the release half-live dropped from 338 to 58 h for MAP−CPT nanoparticles. *In vitro* testing over BT-474 and HER2 human breast cancer cell lines showed that cellular uptake of nanoparticles was enhanced by 70%, compared to non-targeted version, by the incorporation of a single herceptin antibody molecule per nanoparticle. Moreover, CPT was released from MAP-CPT nanoparticles by hydrolysis and nanoparticle disruption by fat. The targeted MAP-CPT nanoparticle system carried

ca. 60 CPT molecules per nanoparticle and showed prolonged plasma circulation with an elimination half-life of 21.2 h and AUC value of 2766 μg h/mL at a 10 mg CPT/kg tail vein injection in female BALB/c nude mice.

Encapsulation of CPT in micelles with a hydrophilic shell of chitosan and a hydrophobic core of poly(p-dioxanone) has been also used to control drug release on the basis of environment pH changes [169]. CPT was incorporated in the core of this system and its release was triggered at pH 7.4, 6.2 and 5.0. *In vitro* drug release studies in HeLa human cervix carcinoma and L929 mouse fibroblast cells demonstrated that the micelles presented a much faster release of CPT at pH 5.0 than at pH 7.4, whereas blank micelles without CPT were found to be nontoxic in both cell lines. The good internalization effect of these micelles was explained by the electrostatic interactions between positively charged micelles and cell membranes.

In another recently published study, an inorganic pH-sensitive nanocarrier was able to deliver different chemotherapeutic drugs simultaneously for a better synergetic effect. This nanoplatform was based on hollow silica nanoparticles sealed with ZnO quantum dots (QDs). Subsequently, CPT was encapsulated in the hollow core through hydrophobic interactions. On the other hand, doxorubicin (DOX) was deposited on the mesoporous shell *via* electrostatics interactions with carboxylate groups incorporated on the mesoporous surface [170]. This cooperative drug loading largely increased drug encapsulation efficacy of both CPT and DOX up to 89.28% and 44.98%, respectively. Drug release was compared at pH 7.4 and pH 5.0, concluding that in acid medium the amount of discharged drugs was higher due to ZnO QDs disintegration.

4.2. Redox-sensitive systems

It is well-known the difference in redox potential between the oxidizing extracellular space and reducing intracellular space, which is related with glutathione (GSH)

concentration. This fact has found application in the building of specific DDSs able to selectively discharge their therapeutic load after cell uptake. Here, drug covalent linking to nanocarriers is mostly carried out by disulfide bridge, which makes possible to design of different sensitive systems. The main concern for *in vivo* application of these systems is the presence of GSH in serum which, despite the fact that its concentration is less than one order below that of cytosol, may provoke some premature release during CPT transit at blood stream [171].

Hybrid micelles subjected to redox-responsive cleavage have been developed by cross-linking CPT-loaded organic units with disulfide bonds [172]. These micelles were obtained *via* coprecipitation of disulfide-containing CPT-poly(tyrosine(alkynyl)-*O*-carboxyanhydride) conjugate and monomethoxy poly(ethylene glycol)-*b*-poly(tyrosine(alkynyl)-*O*-carboxyanhydride), followed by cross-linking of the micellar core *via* click chemistry (Fig. 14). In this case, CPT was rapidly released due to the elevated concentration of cytosolic glutathione that disorganizes micelle structure by disulfide bond cleavage, which is present both in the CPT prodrug and the cross-linker. *In vitro* cytotoxic assays against MCF-7 human breast cancer cells suggested high anticancer activity due to the disassembly of the cross-linked micelle and rapid CPT release.

Also recently, our group implemented a new synthetic strategy by direct coupling of as-synthesized (pyridin-2-yldisulfanyl)alkyl carbonate derivatives of CPT with thiol groups of silica hybrid nanoparticles containing a non-porous core and a mesoporous shell [173]. Upon reaction with thiols in physiological conditions, disulfide bridge cleavage occurs, releasing the naked drug after an intramolecular cyclization mechanism (Fig. 15). Additional incorporation of a fluorophore into particles core facilitated imaging at the subcellular level for the monitoring of uptake and delivery. Confocal microscopy experiments in HeLa human cervix carcinoma cells confirmed that nanoparticles enter

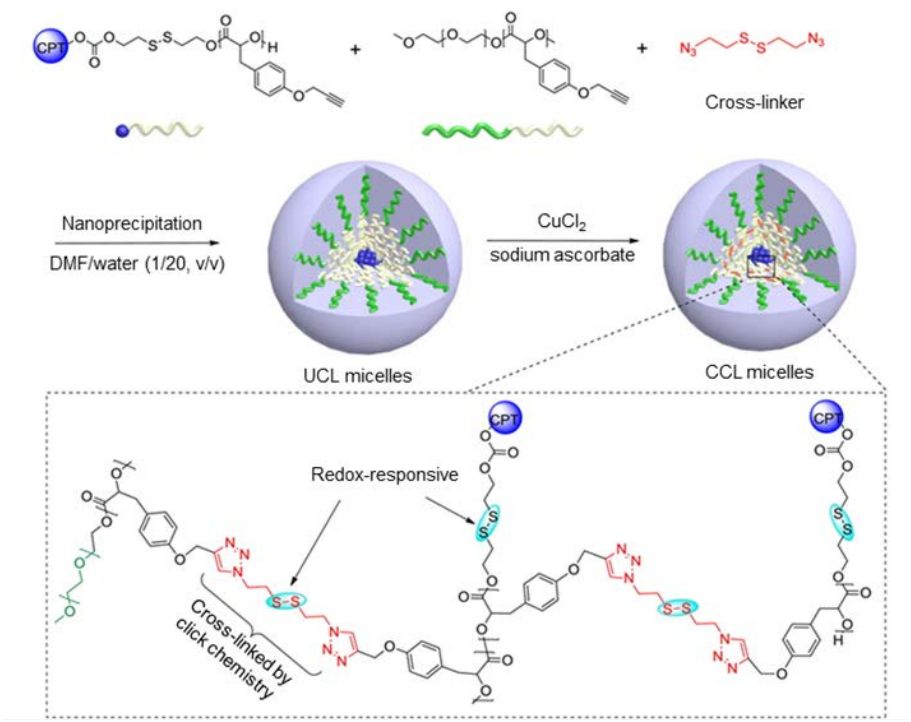


Fig. 14. Schematic procedure for preparation of redox-responsive uncross-linked (UCL) and core-cross-linked (CCL) hybrid micelles. (Reprinted partially with permission from ref. 172. Copyright © 2013 American Chemical Society)

the cells by endocytosis but are able to escape from endo-lysosomes and enter the cytosolic compartment to release their cargo by glutathione reducing activity. Moreover, in a separated study [174], it was shown that prodrug side chain carbon number (*n*) determines material hydrophobic properties and, as a consequence, its stability in aqueous medium and cell uptake kinetics. When *n* value increases, the negative surface charge decreases dramatically due to a shielding effect provoked by hydrophobic ligands, which promotes particle aggregation and favors cell internalization. Furthermore, *n* value also determines the type of products released and, subsequently, the cytotoxic activity. Although full disulfide bridge reduction occurs in all cases within the cell, the subsequent intramolecular cyclization releasing CPT, CO_2 and the corresponding thiolactone only happened quantitatively for *n*=1 (Fig. 15), whereas higher homologues released low CPT quantities (5% for *n*=2 and traces for *n*=3).

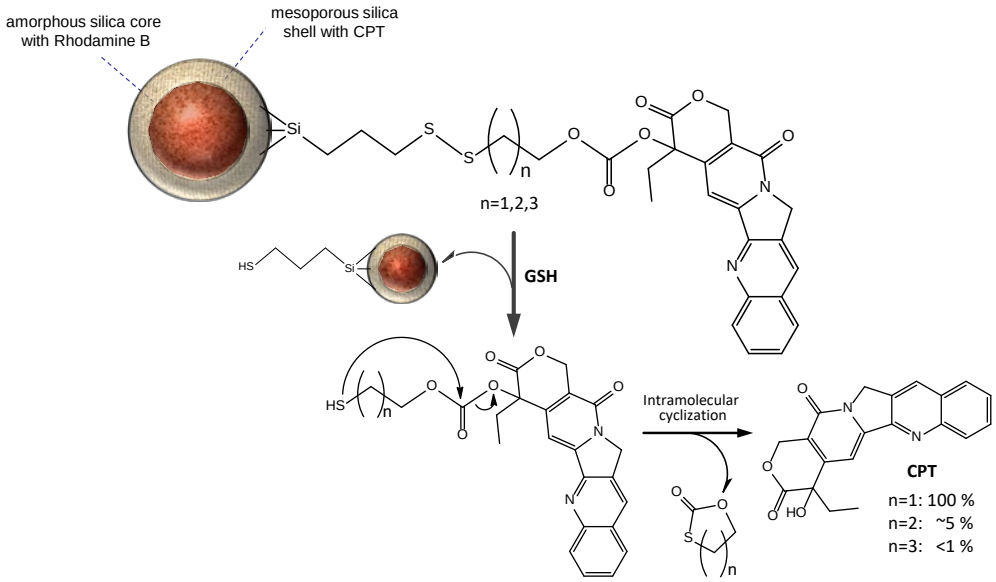


Fig. 15. Redox-responsive nanoplatform for CPT delivery by direct coupling of (pyridin-2-yldisulfanyl)alkyl carbonate CPT derivatives with thiol groups of silica hybrid nanoparticles containing a non-porous core and a mesoporous shell. Upon reaction with thiols in physiological conditions, disulfide bridge cleavage occurs, releasing the naked drug after an intramolecular cyclization mechanism. (Adapted from ref. 173, with permission of The Royal Society of Chemistry)

A supramolecular strategy was reported to directly assemble the hydrophobic CPT molecule to a β-sheet-forming peptide sequence derived from the cysteine-containing au protein through the reducible disulfylbutyrate (buSS) linker, obtaining discrete, stable, well-defined nanostructures with a high and quantitative drug loading [175]. Drug content was precisely controlled using the two amine functionalities of the amino acid lysine to create branching points that allow the attachment of one, two or four CPT molecules, corresponding to respective drug loadings of 23%, 31% and 38%. Depending on the number of CPT molecules in the supramolecular design, the resulting nanostructures can be either nanofibers or nanotubes. Such nanostructures provide CPT protection from the external environment. Release profiles showed that glutathione degraded the designed buSS linker, releasing a mixture of intermediates and CPT. Under tumor-relevant conditions, these drug nanostructures released the active form of CPT and showed *in vitro* efficacy similar to free CPT against MCF-7 human breast cancer cells and 9L and F98L rat gliosarcoma cell lines. In addition, to avoid the formation of

intermediates, the same researchers prepared these CPT-nanostructures using a (disulfanyl)ethyl carbonate linker, that after disulfide bridge reduction by glutathione followed a self-immolative process to release free CPT, improving cytotoxicity over MCF-7 cell line [176]. Furthermore, *in vivo* preliminary experiments, after intratumoral injection with 36% CPT loaded sample over 6-8 week female BALB/c mice bearing subcutaneous CT26 murine colorectal adenocarcinoma, showed higher enhanced retention time of CPT nanotubes in the tumor site than the free molecule, which allows a sustainable release over a long period [177]. Conversely, no improvement in tumor targeting was detected with the nanomedicine in case of administration by tail vein, so the potential of this impressive nanoconjugate as DDS is still to be confirmed.

4.3 Enzyme-sensitive systems

In these drug delivery nanoplatforms, the therapeutic molecule is engaged to nanoparticles by a covalent bond that may be cleavage by enzymatic activity. Enzyme-responsive nanomaterials facilitate the targeting of specific tissue by programming drug release *via* enzymatic digestion of the nanocarrier or linking moiety [178]. The biological recognition of the substrate by the enzyme leads to the selective and specific triggering event to release the cargo, which minimizes toxicological issues related to CPT. In this context, due to the high concentration of carboxylesterases in the cytosol, most of these models have been developed over the basis of an ester bond at 20-OH position between CPT and the nanostructured surface. On the other hand, there are two main shortcomings in the use of enzyme-responsive devices for CPT delivery: i) the presence of carboxylesterases in plasma may motivate some nonspecific release outside the tumor cells, and ii) the binding of CPT to a nanoparticle surface can limit the accessibility to enzyme active site due to steric effect, slowing down the release process, which reduces the cytotoxic activity [179].

In a singular approach, CPT molecules were directly grafted to carboxyl groups of poly(L-glutamic acid) (CPT-PGA) through ester bond [180]. Subsequently, a supramolecular nanoparticle system was obtained by conjugation of CPT-PGA with several polymeric derivatives (Fig. 16). This system released CPT under physiological conditions by means of esterase-mediated hydrolysis. Cell viability assays in MCF-7 human breast cancer cells indicated nanoparticle delivery system IC_{50} was 4 times that of the free drug, probably due to the slow release of CPT from polymer. However, a positron emission tomography study with ^{64}Cu-labeled nanoparticles in C57Bl/6 mice bearing Lewis lung carcinoma tumors revealed significant tumor specific uptake of nanoparticles. Moreover, in tumor reduction/inhibition studies these nanomedicines showed much higher efficacy than the free drug, delaying tumor growth with no visible body weight or any other side effect.

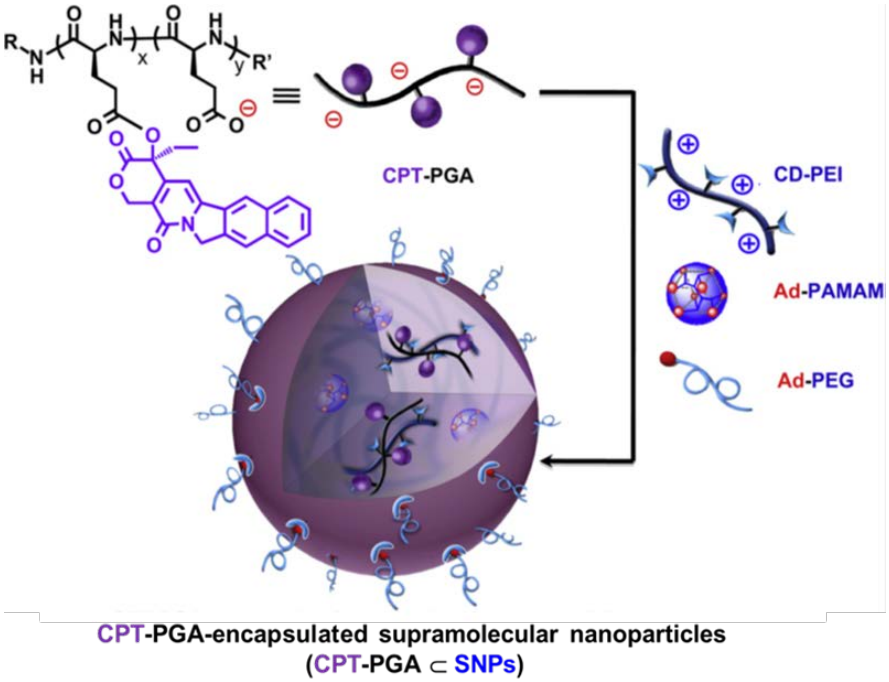


Fig. 16. Schematic representations of the self-assembly and synthesis of supramolecular nanoparticles by conjugation of CPT-grafted poly(L-glutamic acid) with different polymeric derivatives. Legend: Ad-PEG=poly(ethylene glycol); CD-PEI= cyclodextrin-poly(ethylenimine); Ad-PAMAM=poly(amido)amine (Reprinted from ref. 180, Copyright © 2012, with permission from Elsevier)

Conversely, CPT conjugation to nanoparticles by ester bond is usually performed in two steps. First, a prodrug is prepared by esterification with a short linker and then, this

prodrug is covalently linked to a supramolecular structure. For instance, to incorporate CPT into PAMAM, CPT was firstly substituted at 20-OH position with glycine through ester bond and, afterwards, the prodrug was reacted with PAMAM G4, to give PAMAM-CPT [181]. This material was stable in plasma and the presence of proteolytic enzymes such as cytosolic esterases promoted conjugate cleavage and CPT release. Cell internalization and *in vitro* assays of this nanomedicine in HTC-116 human colorectal cancer cells indicated a high cytotoxic effect due to nuclear fragmentation and formation of apoptotic bodies.

As a different model, our group recently prepared a hybrid organic-inorganic silica nanoplatform for CPT delivery based on enzyme-triggered release. For this sake, CPT was covalently linked to nanoparticles through an ester bond with the 20-hydroxy moiety, in order to stabilize its lactone ring and to avoid unspecific release of the drug [179]. The obtained material was highly stable in plasma, with less than 5% release of the cargo at physiological pH. Cell internalization and *in vitro* efficacy assays against HeLa, U87-MG human primary glioblastoma cell line, HCT-116 human colon carcinoma and HT-29 human colorectal adenocarcinoma cells, demonstrated that nanoparticles carrying CPT (SNP-CPT) entered cells *via* endocytosis and the intracellular release of the cargo induced cell death with half maximal inhibitory concentration (IC_{50}) values and cell cycle distribution profiles similar to those observed for the naked drug. Furthermore, *in vivo* biodistribution, therapeutic efficacy and biocompatibility of the SNP-CPT were evaluated in hsd:athymic nude-Foxn1 mice bearing HT-29.Fluc subcutaneous tumors, showing that, although SNP-CPT tended to accumulate in organs of the reticulo-endothelial system, nanoparticles delayed the growth of subcutaneous tumors while significantly reducing the systemic toxicity associated to CPT administration (Fig. 17).

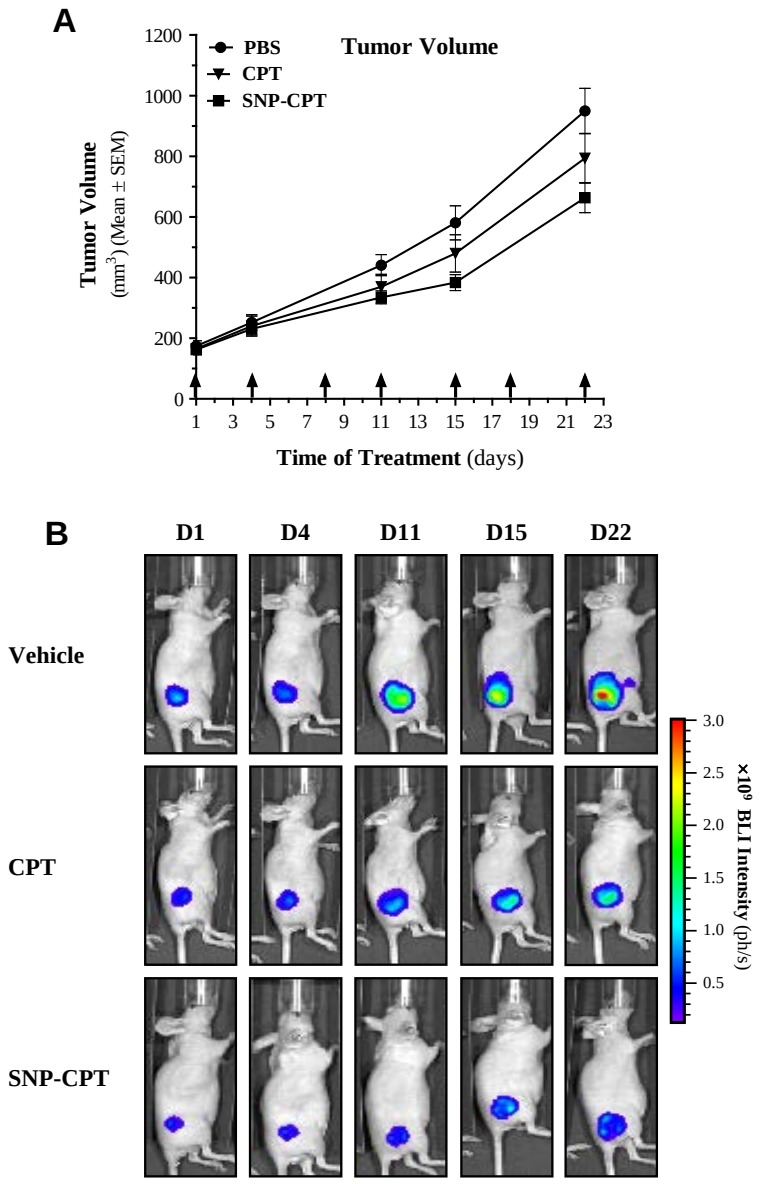


Fig. 17. Tumor growth delay in hsd:athymic nude-Foxn1 mice bearing HT-29.Fluc subcutaneous (s.c.) tumors. (A) Comparative analysis of the localized *s.c.* growth of the HT-29.Fluc tumors untreated (PBS) and treated (CPT or SNP-CPT) in hsd:athymic nude-Foxn1 mice by external measurement (caliper) of tumor volume. (B) *In vivo* monitoring of a representative mouse from each treatment group shows radical tumor growth reduction with regards CPT and the control group. (Reprinted partially from ref. 179, Copyright © 2011, with permission from Elsevier)

4.4. Light-sensitive systems

In addition to chemically-triggered delivery systems, remote activation of nanoparticles by light for biomedical applications has become an important field of research [182]. This type of stimuli-responsive nanoplatforms present a component sensitive to ultraviolet

(UV), visible (Vis) and/or near infrared (NIR) light which is able to activate drug release under irradiation. As an exogenous stimulus, the main advantage of light over endogenous processes is the possibility to accurately modulate stimulation that controls drug release. In this sense, light is not only noninvasive but also controllable both spatially and temporally, allowing for greater safety and specificity [183]. Conversely, low penetration ability and predominant use of UV and visible light limit the clinical potential of these systems. So far, many different systems have been described, involving photocleavage, photodissociation, photoisomerization, photorelease, photothermal, photo-plasmonic heating, and up-conversion photoisomerization [184].

In a pioneering work, a nanoimpeller-controlled mesostructured nanoparticles was developed to deliver and release CPT into living cells upon light activation [185]. These light-triggered MSNs were functionalized with azobenzene moieties in the pore interiors, with one end attached to the pore walls and the other end free to undergo photoisomerization. The impeller in the nanopores trapped drug molecules in the dark, and release them in response to UV light (413 nm) excitation (Fig. 18). *In vitro* studies in PANC-1 human pancreatic and SW480 human colon cancer cell lines showed the photoresponsive behavior of the impeller inside of cells. After 3 h of incubation in the dark, the cells were irradiated with ~0.1 W cm^{-2}, 413 nm light, for various excitation times (0 to 10 min). Cell death was induced under photocontrol. In the absence of light excitation, the CPT remained in the particles and cells were not damaged. However, illumination promptly expelled CPT from the particles, causing cancer cell apoptosis, which is demonstrated by nuclear fragmentation and chromatin condensation.

Unfortunately, the application of UV/Vis light in biomedical studies is mostly limited to proof-of-concept, because irradiation at this wavelength can damage cells and does not penetrate deep inside the tissues. However, using two-photon excitation (TPE) in the NIR region instead of UV/Vis light leads to better tissue penetration, lower scattering

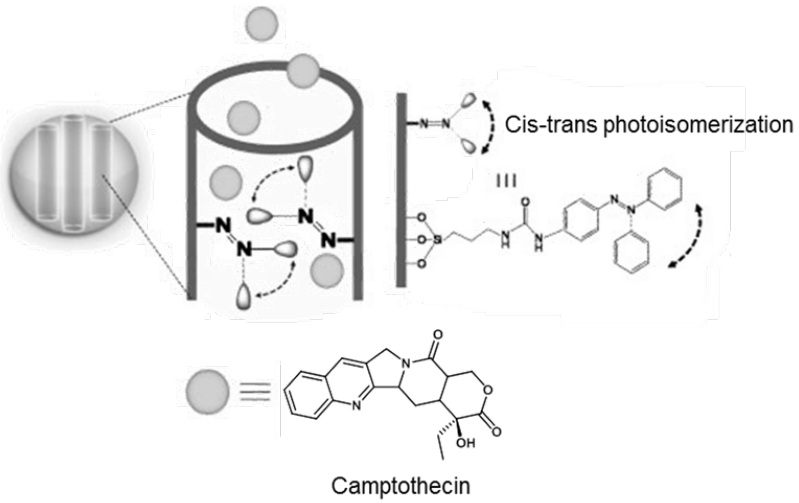


Fig. 18. Schematic illustration of the light-activated mesostructured silica nanoparticles functionalized with azobenzene derivatives in the pore interiors. (Adapted with permission from ref. 185. Copyright © 2008 John Wiley and Sons)

losses and three-dimensional spatial resolution. For this sake, the former nanoimpeller-controlled MSNs device was functionalized with a high absorption cross-section two-photon fluorofore, suitable for Forster resonance energy transfer (FRET) to photoizomerize azobenzene moieties in the NIR region [184]. The two-photon absorption properties of the fluorophore were retained in the materials and no decrease of cross-sections $\sigma 2$ was noticed after encapsulation. The nanoimpeller groups pending in the porous framework allow the physical entrapment of CPT, which is then kicked out of the pores by two-photon-triggered photoisomerization. Therefore, the nanoimpellers were loaded with CPT and then screened under TPE in MCF-7 human breast cancer cells. Cells were incubated with nanoparticles and further irradiated in at very short time with a focused laser beam (input 3 W, output 900 mW cm^{-2}). The MTT assay was performed two days after irradiation. Nanoimpellers loaded with CPT and high fluorofore/azobenzene ratio significantly increased cancer cell death under TPE in comparison to no irradiation.

Another representative light-responsive prototype was developed as a self-deliverable form of nucleic acid-CPT nanostructure (DNA-CPT) that is composed almost entirely of payload molecules [186]. To tackle this strategy, three CPT molecules were attached to

a phenol group connected to a photolabile 2-nitrobenzyl ether moiety. This photolabile group was linked to a DNA strand to obtain an amphiphile, which self-assembled in DNA-CPT nanostructure. Under UV irradiation (365 nm), the 2-nitrobenzyl was cleaved, releasing the DNA from the conjugate and leaving a decapped self-immolative drug core, which, afterwards, underwent a rapid, spontaneous and irreversible degradation process to release free CPT molecules. *In vitro* efficacy assays in SK-BR-3 human breast cancer cell line confirmed that cytotoxicity of DNA-CPT nanostructure increased significantly by light triggering, achieving IC_{50} values similar to those observed with the naked drug.

In a different strategy, the surface of MSNs may be modified in order to deposit a hydrophobic layer able to monitor drug delivery by adjusting the wetting of particle surface. In this context, a light-sensitive release system based on a hybrid with MSN core and spiropyran and perfluorodecyltriethoxysilane coating was built [187]. Particles are protected from being wetted by water at the optimal ratio of spiropyran to fluorinated silane (0.249:1), which successfully inhibits drug release. Upon irradiation with UV light (365 nm), the conformational conversion of spiropyran from a “closed” state to an “open”

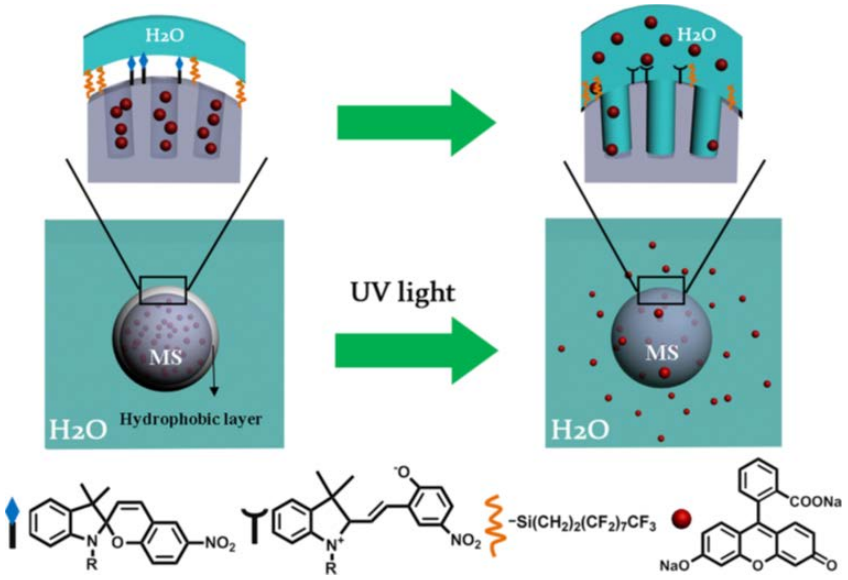


Fig. 19. Schematic presentation of the light-sensitive release system based on mesopororus silica nanoparticles surface functionalized with spiropyran and perfluorodecyltriethoxysilane in an optimal ratio. Under UV irradiation at 365 nm, spiropyran was converted to the hydrophilic form, which resulted in the wetting of surface of MS and the release of trapped molecules diffusing from the penetrated water. (Reprinted with permission from ref. 187. Copyright © 2014 American Chemical Society)

state caused the surface to be wetted, leading to the release of encapsulated CPT from the pores (Fig. 19). *In vitro* experiments in EA.hy926 human somatic cell hybrid and HeLa human cervix carcinoma cells demonstrated an accurate drug controlled release. Here, CPT loaded nanoparticles (10 mg/g) were incubated in cells for 24 h and then, upon UV light irradiation a noticeable loss of cell viability was observed with regards to non-irradiated cell cultures or with no nanoparticles.

4.5. Magnetic-sensitive systems

Magnetic gradients may be used to target therapeutics to specific tissues, while alternating magnetic fields produce frequency-dependent effects at the nanoparticle level. This type of nanocarriers implicates para-magnetic or super-paramagnetic materials which are embedded into organic polymeric moieties, allowing the release of the drug under an external magnetic stimulus. The inorganic component is designed to impart responsivity to external magnetic fields, both static and dynamic, in order to impose a remote magnetic control of drug cargo release. Furthermore, soft matter architectures provide a very convenient structural scaffold for insertion of therapeutic principles [188]. Nevertheless, as commented before (section *3.2*), the most serious concern about the use of magnetic nanoparticles within therapeutic formulations is their biocompatibility, especially in the long term. In particular, their stability when introduced within the body is a crucial aspect, as the proper dispersion and circulation of nanoparticles strongly affect drug release kinetic, accumulation, and toxicity. Actually, SPIONs are the only engineered inorganic nanoparticles approved for human use.

One of the most illustrative drug delivery models of hybrid magnetic materials is based on MSN pore capping with iron oxide nanoparticles [189]. This may be preferentially done through chemical bonding. For this sake, MSNs surface was funtionalized with 3-

aminopropyltrimethoxy silane groups. Afterwards, CPT was filled into the pores and these were covalently capped through amidation of the 3-aminopropyl groups at surface with meso-2,3-dimercaptosuccinic acid functionalized superparamagnetic iron oxide nanoparticles [190]. The Fe_3O_4 nano-caps formed a dense and uniform layer tightly bound to the MSN surface (MSN@Fe_3O_4). Under magnetic stimulus, the cumulative drug release was very quick, and prolonged even when the stimulus was removed, due to detachment of some Fe_3O_4 nanoparticles (Fig. 20). Such a magnetically-induced removal of the nano-caps implies a cleavage of the chemical bond between the Fe_3O_4 nanoparticles and silanized MSNs. The energy induced from the magnetic stimulus over various timespans is sufficiently large to cleave the chemical bond, resulting in an effective removal of the nano-caps. Actually, the rate of nano-cap removal and drug diffusion are a function of the magnetic energy. Then, cytotoxicity studies in A549 human lung adenocarcinoma epithelial cell line by MTT assay, indicated that nano-Fe_3O_4-

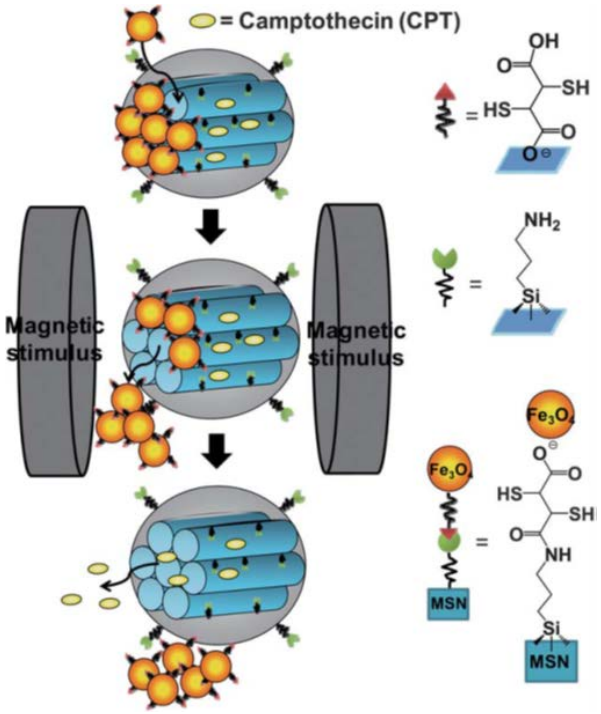


Fig 20. Schematic illustration of the synthesis and structure of mesoporous silica nanoparticles capped with iron oxide nanoparticles. The structure was obtained by capping mesoporous silica nanoparticles (MSN) with monodispersed Fe_3O_4 nanoparticles through chemical bonding. Under magnetic stimulus, some of the Fe_3O_4 nanoparticles were removed from the surface of the MSN, and CPT was released. On the right, a brief illustration of all different ligands used in the synthesis of this material. (Reprinted from ref. 190 with permission of The Royal Society of Chemistry)

capped MSN effectively constrained the CPT from free elution and were well tolerated, resulting in a cell viability of more than 80%. However, upon magnetic stimulus, about 42% of the cells were killed, and the major cause of cell death was the CPT released from the MSN@Fe_3O_4.

Magnetic stimulus can also be used to accelerate drug release from core-shell hybrid nanoparticles constructed by self-assembly of iron oxide in the presence of polyvinyl alcohol. Then, CPT was incorporated to the organic component of the core, followed by coating with a thin layer of silica to eliminate undesirable drug leakage [191]. Here, the operation of an external magnetic stimulus allows a pulse-type drug release to be readily achieved in a real time responsive manner without undesirable delays in dosing accuracy. The intracellular release of CPT from nanocarriers in A549 cells was stimulated by high frequency magnetic field (HFMF, 2.5 kA m^{-1}, 50 kHz) for 30-120 s. Upon magnetic stimulus, CPT release profile for the nanocarriers in different eluting media indicated that the rate of drug diffusion was considerably increased, which is due to thermally induced disruption of particle nanostructure [192]. Subsequently, cells were incubated for 18 h, and the anticancer effect was measured by MTT assay. It was found that CPT IC_{50} value measured on A549 human lung adenocarcinoma epithelial cells was around five times lower than that of the free drug when CPT was loaded into magnetically sensitive nanocarriers and released intracellularly. Moreover, a similar model was performed by preparation of embedded Fe_3O_4 nanoparticles in a PLGA core containing CPT, protected by a lipid–polymer hybrid shell [193]. *In vitro* testing of these drug delivery capsules in MT2 mouse breast cancer cells showed significantly suppressed cancer cell growth upon magnetic field activation.

4.6. Other stimuli-responsive systems for camptothecin delivery

In addition to the different stimuli-responsive systems described, in some recent studies other analogue entities have been proposed for CPT delivery and controlled release under endogenous or exogenous stimulation. We here summarize some of most advanced approaches.

Thermo-responsive nanomaterials are a kind of adaptive nanoparticles sensitive to environmental temperature changes that may help to monitor spatial delivery of a therapeutic payload. Under pathological local high temperature records (for instance, those reported in inflammation and cancer) these systems undergo a phase transition and conformational change that compromise the integrity of the nanostructure and activate payload release. At this point, polymers can be designed in such a manner so that this phase transition, which is termed as the lower critical solution temperature (LCST), takes place at around 37 ºC. Under LCST the hydrogel nanopreparation is soluble for *in vivo* injection, but becomes insoluble and aggregated in the tumor regions heated above its LCST, leading to local drug discharge [194]. In this context, CPT thermo-sensitive DDSs can be prepared based on spherical core-shell nanoscale micelles. Firstly, two kinds of thermo-sensitive poly(N-isoproplacrylamide) (PNIPAM) four-armed star multiblock copolymers were synthesized by atom transfer radical polymerization. Then, the multiblock copolymers could spontaneously assemble into regular spherical core–shell nanoscale micelles of about 100-120 nm diameter and LCST of about 33-35 ºC [195]. Drug release was thermo-responsive, accompanied by temperature-induced structural changes of the micelles. At 37 ºC, CPT release from micelles is clearly quicker than at 25 ºC (respectively, 74 and 53% at 48 h) due to temperature-induced structural alterations of multiblock copolymers. This accelerated drug release above the LCST is correlated with the enhanced hydrophobicity of PNIPAM shells, which leads to micelle shrinkage, and makes CPT diffuse out quickly. *In vitro* cytotoxicity studies carry out by MTT assay over MDA-MB231 human breast cancer cell

line, demonstrated slightly lower activity for the CPT-loaded micelles than for the free CPT, probably due to time-consuming CPT release from the micelles.

Ultrasounds represent another effective method for attaining spatio-temporal controlled release at the target cells, preventing side effects to healthy tissues. Ultrasound waves can trigger drug through the thermal and/or mechanical effects generated by cavitation phenomena or radiation forces. Here, physical forces associated to cavitation can induce nanocarrier destabilization, drug release and transient increase in vessel permeability, leading to the cellular uptake of therapeutic molecules. Acoustically active perfluorocarbon nanoemulsions have been proposed for CPT encapsulation, in order to circumvent its well-known bioavailability issues as insolubility, instability and toxicity [196]. The nanoemulsions were firstly prepared with perfluoropentane, perfluorohexane and coconut oil as the core of the inner phase, which incorporates CPT molecules. Then, a stabilizing coating made of phospholipids and/or Pluronic F68 was applied, obtaining a mean droplet diameter of 220-420 nm. For the study of ultrasound influence on CPT release the nanomulsion was exposed to ultrasound using a 1 MHz probe with a 1.5 W/cm^2 intensity for 2 h. However, CPT released from the nanoemulsions was limited, with less than 15% of the drug being discharged over 48 h. Moreover, it was found that droplet diameter had an influence in release rate, as the amount of exposed surface decreased for bigger droplets, which slows down CPT liberation. Furthermore, in order to simulate parenteral administration conditions, plasma was added to the nanoemulsions and, next, sonication with ultrasound energy was used to stimulate the liquid-to-gas transition of perfluorocarbons [197]. As the droplets became gas bubbles, they began to oscillate or resonate and, eventually, bubbles were destroyed and provoked a local drug release. Unfortunately, no biological study is reported with this interesting CPT controlled release model.

Recently, glucose-responsive materials have attracted research interest due to their potential application as DDSs. Most of these systems have been developed as platforms

for glycemia control in diabetes mellitus patients. Among them, the most studied model is that based on phenylboronic acid [198]. In aqueous medium, phenylboronic acid holds an equilibrium between an uncharged form and a charged form. The first one is hydrophobic but the second form is hydrophilic. This last is able to form a stable phenylborate with cis-diol compounds, like glucose, which shifts the equilibrium in the direction of increasing the hydrophilic form. Usually, this shift is enough to increase the swelling degree of crosslinked nanogels or to disintegrate self-assembled micelles, causing the entrapped payload to be released into solution. This provides a way for designing polymeric carriers for self-regulated delivery not only of insulin but also of other therapeutic agents. For the purpose of CPT delivery, hybrid nanoparticles with iron oxide and mesoporous silica were loaded with the drug and further capped with a phenylboronic-dextran conjugate [199]. In a further step, CPT was encapsulated in silica mesopores followed by incubation with dextran (Mw 6000 and 100000), which bounded with phenylborate linker *via* glucose monomer 1,2-diol groups (Fig. 21). Consequently, dextran closes the surface of pores, preventing drug release. CPT release mechanism is based on the competition between soluble glucose and dextran for binding

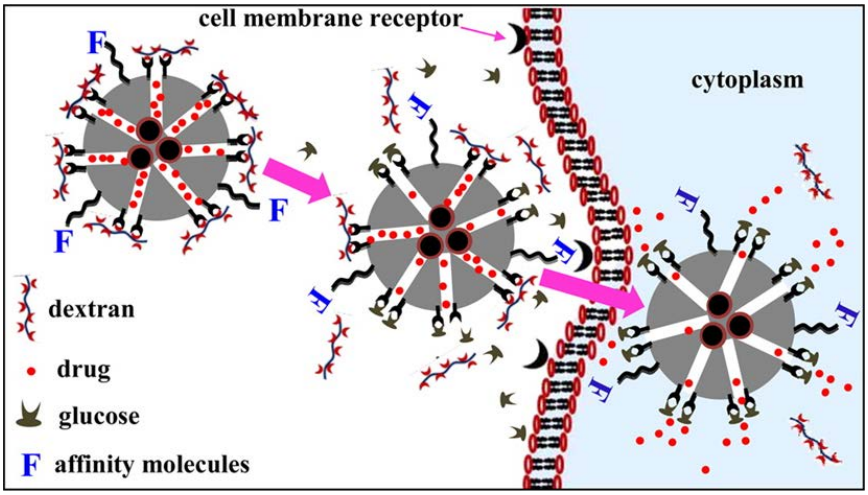


Fig. 21. Schematic procedure for preparation of glucose-responsive hybrid nanoparticles with iron oxide cores and dextran-gated mesoporous silica shell. The design involves the synthesis of magnetic mesoporous silica nanoparticles (MMS) functionalized with phenylboronic acid and folate. Then, CPT was loaded inside the pores of MMS, the outside of pores was closed by dextran *via* binding with phenylboronic acid. Dextran-gated pores were opened for drug release in the presence of glucose that competes binding with phenylboronic acid. (Reprinted with permission from ref. 199. Copyright © 2014 American Chemical Society)

phenylboronic acid. Glucose has one 1,2-cis diol group that binds strongly to phenylborate linker, removing the larger size dextran from pore surface. *In vitro* results over HeLa human cervix carcinoma cell line showed a significant reduction of cell viability in glucose containing RPMI cell culture medium, but no effect in the absence of glucose, showing that drug loaded particle offers glucose concentration-dependent cell viability.

To end this section, we can summarize that stimuli-responsive systems are able to deliver CPT to tumor tissue and cells with minimum (or meaningless) premature release, imposing an efficient drug discharge inside cells under a specific stimulus. This is particularly important in the case of very cytotoxic molecules like CPT, as these DDSs increase anti-tumor activity and minimize side effects. It must be taken into account that for most DDSs, less than ~5% of the injected dose reaches the tumor site [161]. In this context, the design of nanocarriers sensitive to exogenous or endogenous stimuli represent a safe alternative to regular chemotherapy with CPT structural analogues. The main concern with most of these systems, for translation from the bench to the bedside, is related to their chemical complexity, as most of them are hybrid materials with organic and inorganic components, and several-step synthesis protocols. This hampers not only the scaling-up of their synthesis, but also their degradability and biocompatibility. Moreover, the ability of these models to be sensitive to discrete variations of pH, redox potential or enzyme activity is not always straightforward to achieve, whereas the scope of externally applied stimulus is strongly depends on the penetration depth ability. Nevertheless, some of these systems are on the way towards achieving clinical application, as it will be shown below.

5. Clinical testing

One of the most disputable arguments again nanomedicines is that, despite the strong publication statistics over the last decade, very few drugs have reached the commercial market yet [79]. Although it is accepted that nanocarriers improve solubility, efficacy and

biodistribution, while decreasing adverse side effects of very cytotoxic drugs as CPT, strict regulations concerning their use in human beings have hampered, so far, a quicker translation of these therapeutic platforms into the clinical stage. At this point, it must be taken into account that nanocarriers are evaluated for each of their components within the formulation, and aspects like colloid stability in biological fluids, low immunogenicity and long-term toxicity may not always be assessed. Moreover, the necessary refining of the delivery process performance is yet to be achieved [200]. In this context, only a few organic polymers have been approved by the Food and Drug Administration (FDA) as CPT delivery systems for cancer therapy. Below we describe briefly some of the most representative examples as well as their current position at the pipeline. All these cases have been compiled in Table 2. We accept that some of these DDSs might not form solid nanoparticles in real *in vivo* conditions [201], but in all cases, the methods followed for material preparation, drug administration, biodistribution profile study and therapeutic validation are fully comparable.

CRLX101 (formerly IT-101) is a pharmaceutical polymeric nanoparticle developed at Calando Pharmaceuticals, Inc. composed of a cyclodextrin-PEG copolymer covalently linked to CPT, and was designed to enhance drug solubility and biodistribution [117]. The antitumor drug was released by hydrolysis of the ester bond between the cyclodextrin and CPT hydroxyl group at C20 position. With regards to irinotecan, this compound showed improved cytotoxicity against MDA-MB-231 human breast cancer cells, Panc-1 human pancreatic cells and HT29 human colorectal adenocarcinoma cell line [117,202], entering Phase Ia trial for the treatment of solid tumors over 10 patients in 2006. Also, a combined Phase Ib/IIa trial over 62 patients with advanced solid malignancies demonstrated encouraging safety, pharmacokinetic, and efficacy [203]. This preparation was tolerated with no evident side effects. Consequently, a Phase II trial was started in 2008 for therapy of 29 ovarian cancer patients and, later on, in 2011, a Phase II trial was launched over 157 patients with non-small cell lung cancer, in order

to assess the activity and safety of CRLX101. In addition, comparative preclinical studies suggested that CRLX101 behavior in animal models could be the same than in humans [204]. Actually, we should consider that, although nanomedicine accumulation in solid tumors by EPR effect is quite rationalized for animal models of human disease, these models poorly represent human tumors. Nevertheless, in a very recent study over 9 gastric tumor patients consisting of collecting tumor and nonneoplastic tissue biopsies 24-48 h after CRLX101 administration, evidence indicated an intact deposition of nanoparticles in tumor tissue in five of these 9 patients, whereas no CRLX101 trace was found in nonneoplastic tissue. Moreover, in all cases, sufficient CPT concentration reached the tumor to cause down-regulation of topoisomerase I and carbonic anhydrase IX [205].

CT-2106 is a CPT-poly-L-glutamate conjugate developed to improve the solubility and prevent CPT lactone ring cleavage. In this supramolecular system, CPT binds the glutamate polymer through esterification at 20-OH. In preclinical models, CT-2106 showed antitumor activity against multiple tumor cell lines, such as B16 murine melanoma cells and H322, H460 and H1299 human lung cancer cells, and retained substantial anti-tumor activity in syngeneic (over C57BL/6 mice) and xenogeneic (over female nude mice) tumor models [206]. CT-2106 Phase I trial was started by Cell Therapeutics, Inc. in 2003. A dose escalation study was carried out over 24 patients with different advanced solid tumors [207]. The only serious related toxicity was grade 4 hypersensitivity in a patient with a history of hypersensitivity to irinotecan. Furthermore, Cell Therapeutics, Inc. extended this Phase I trial in 2006. For this sake, 26 patients with advanced solid tumors (50% melanoma), were intravenously weekly administered for three consecutive weeks during a 28-day cycle [208]. Dose limiting toxicities were thrombocytopenia and fatigue. The pharmacokinetic profile of conjugated CPT showed rapid urinary excretion, and similar terminal half-life than free drug. 25 patients were assessable for response, but no objective responses (complete or partial) were noted.

Only 3 patients had stable disease, one breast cancer patient, one histiocytoma and one melanoma, whereas the rest exhibited progression of the disease. Overall, this trial provided a strong indication that CT-2106 may have the ability to substantially improve the toxicity profile of standard CPT.

XMT-1001 is a water soluble macromolecular conjugate (Mw~70 kDa) synthesized by tethering CPT by hydroxyl group at C20 position to a hydrophilic, biodegradable polyacetal polymer, poly(1-hydroxymethylethylene hydroxymethylformal), also called PHF or Fleximer® [209]. This compound was designed to improve efficacy and toxicity with regards to CPT, and showed higher antitumor efficacy compared with irinotecan against HT-29 human colon carcinoma xenografts [210]. CPT discharge involves a dual-step release mechanism: first, XMT-1001 follows an intramolecular transacylation releasing two CPT prodrugs, camptothecin-20-O-(N-succinimidoglycinate) (CPT-SI) and camptothecin-20-O-(N-succinami-doyl-glycinate) (CPT-SA) and, then, these prodrugs hydrolyze to yield CPT. A Phase I study with XMT-1001 was lauched in 2007 by Mersana Therapeutics, Inc. to determine the maximum tolerable dose (MTD), and to assess the safety and pharmacokinetics of XMT-1001 and its release products. Then, a total of 24 patients with small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC) received XMT-1001 by intravenous infusion every 21 days [211]. No drug related serious adverse events were observed during treatment, whereas several patients showed promising evidence of clinical activity, including tumor shrinkage and stable disease for several weeks. Also, preliminary pharmacokinetics findings confirm the formation of release products (CPT-SI, CPT-SA, and CPT) in plasma. Subsequently, a Phase Ib extension study of XMT-1001 was initiated in 2011 over 11 patients (10 with NSCLC and 1 with gastric cancer), aiming at fully exploring the broad anti-tumor potential of this formulation, but no further results have been reported yet.

Table 2. Nanomedicines of CPT under clinical investigation.[a]

Compound	Formulation	Clinical trial	Number of patients	Pathology	Dosis regimen	MTD[b]	Toxicity	Ref.
CRLX-101	Cyclodextrin-PEG copolymer	Phase Ia	10	Advanced Gastric, Gastroesophageal, or Esophageal Squamous or Adenocarcinoma	60 minute IV infusions on days 1, 8, and 15 of a 28 day cycle	30 mg/m^2/month	Pancytopenia	[202]
		Phase Ib/IIa	62	Solid malignances	60 min IV infusion for 3 consecutive weeks every 28 days	15 mg/m^2 IV every 2 wks	Myelosuppression, grade 3/4 neutropenia and fatigue.	[203]
		Phase II	29	Ovarian Cancer Fallopian Tube Cancer Primary Peritoneal Cancer	IV infusion once every 14 days. Each cycle is 28 days	15 mg/m^2 IV every 2 wks	G3 vasovagal reaction G3 pneumonia G3 pulmonary embolism	[117]
		Phase II	157	Non-small cell lung cancer	15mg/m^2 IV every other week	15mg/m^2 IV every 2 wks	Fatigue/asthenia, dypsnea	[117]
CT-2106	Poly(L-glutamic acid) polymer conjugate	Phase I	24	Adult solid tumors	10 min IV infusion every 3 wks	75 mg CPT-2106/m^2	Neutropenia and thrombocytopenia, febrile neutropenia, grade 2 hematuria, stomatitis	[207]
		Phase I	26	Advanced solid tumors (50% melanoma)	10 min IV infusion weekly x 3 every 4 wks	25 mg CPT-2106/m^2/week	Grade 3 fatigue and grade 3 and 4 thrombocytopenia, grade 2 hematuria	[208]
XMT-1001	Polyacetal poly(1-hydroxymethylethylene hydroxymethylformal)	Phase I	24	Small and non-small cell lung cancer	1-20.5 mg CPT eq/m2 IV infusión once every 3 wks	113 mg CPT (eq)/m^2	Myelosuppresion, diarrhea	[211]
		Phase Ib	11	10 with non-small cell lung cancer and 1 with gastric cancer	IV infusion once every 3 wks	initially 113 mg CPT (eq)/m^2	---	[209]
EZ-246	Pegylated CPT conjugate	Phase I	37	Solid malignances (15 with colorectal cancer)	1 h IV infusion once every 3 wks	7000 mg/m2 (1.67% CPT weight load)	Myelosuppression, bladder toxicity at 4800 mg/m^2	[213]
		Phase II	35	Advanced and metastatic adenocarcinomas of the stomach gastroesophageal junction	1 h IV infusion once every 3 wks	3240 mg/m^2	Neutropenia, thrombocytopenia, anaemia	[214]
MAG-CPT	N-(hydroxypropyl)methacrylamide polymer	Phase I	23	Metastatic, refractory solid tumors (6 with colorectal cancer)	30 min IV infusion once every week	100 mg CPT/equiv./m^2 every 4 wks	Myelosuppression, grade 2 dysuria/hematuria	[218]

[a] *Additional information may be found at www.clinicaltrials.gov.*
[b] *MTD: Maximun tolerated dose*

Conversely, EZ-246 (also known as pegamotecan) is a covalent conjugate of CPT with poly(ethylene glycol) (Mw~40 kDa), developed by Enzon Pharmaceuticals. In preclinical *in vivo* studies, this compound showed higher activity than irinotecan and topotecan against human tumor xenografts (including colon, lung, breast and pancreatic origin) [212]. Then, in a Phase I trial of this formulation, 37 patients with different solid malignancies (15 with colorectal cancer) were administered by intravenous infusion for 1 h every 3 weeks. Antineoplastic activity was observed in 2 patients, but significant toxicity was associated to the treatment, mostly neutropenia and genitourinary toxicity [213]. Actually, free CPT accumulated slowly in plasma, with a dose proportional pharmacokinetics, which forced to reduce the dose limiting toxicity in 6 patients. Subsequently, a Phase II trial was started at 2004 over 35 patients with advanced and metastatic adenocarcinomas of the stomach and gastroesophageal junction [214-215]. Partial antitumor response was observed in 5 subjects, with a median time to progression of about 12 weeks, and median overall survival of 38 weeks. However, severe toxicity, including neutropenia, thrombocytopenia, fatigue, nausea, vomiting and anorexia, appeared again in some patients, showing the need of further studies, maybe combined with other active agents.

Finally, MAG-CPT (also known as PNU 166148 and mureletecan) comprises CPT linked to a water-soluble polymeric backbone methacryloylglycynamide (Mw~18 kDa), developed by Pharmacia. Therefore, CPT derivatives were synthetized by conjugation of a N-(2-hydroxypropyl)methacrylamide copolymer precursor with modified CPT at the C20 α-hydroxy with glycine [216]. These conjugates were designed primarily to improve the clinical delivery of CPT, and utilized a hydrolytically labile spacer able to liberate drug intratumourally in a pH and enzyme-dependant manner. Preclinical studies of MAG-CPT showed that this compound was active in colon, stomach, pancreas, ovary, breast, lung, and melanoma xenograft models [217]. Then, a Phase I study over 23 patients with metastatic, refractory solid tumors (6 with colorectal cancer) was carried out in 1999

[218]. Unfortunately, no objective antitumor responses were seen, and severe drug-related toxicity effects were reported, as myelosuppression, neutropenic sepsis and diarrhea. Also, one patient died after the first treatment cycle at the maximum dose. Based on the results of the Phase I studies and the intratumoural kinetic study, Pharmacia made a strategic decision to discontinue further clinical development of MAG-CPT [219].

6. Conclusion and future directions

CPT is one of the most active molecules for cancer treatment, but its particular limitations as poor solubility, lack of stability of the lactone ring and strong toxicity, have encouraged the development of different delivery forms in order to achieve a safe approach. Here, it is a fact that current cancer treatment cannot be tackled as the administration of a single therapeutic molecule but, instead, the new target is personalized medicine, which selects the appropriate drug combination and dosing schedules for individual patients.

In this context, despite the fact that irinotecan and topotecan present improved pharmacokinetic parameters, and that they are the only commercial CPT derivatives approved by the FDA, therapies merely relying on single, small molecules seem now out of step, mostly due to their limited efficacy and unacceptable toxicities. For this reason, huge emphasis is being placed on developing new DDSs which are able to simultaneously deliver CPT and other active agents, as well as incorporating imaging moieties that may ease the diagnosis process. Most specifically, future seems good for those systems which are able to selectively discharge the drug at the target cells under an specific stimulus, with no previous release. Stimuli-responsive preparations provide a dramatically better spatial and temporal control over drug release, which is crucial for delivery of the very cytotoxic CPT.

Although no CPT nanomedicine has achieved commercial form, there are several organic polymer compositions currently under different phases of clinical trials. In all cases, there is a significant increase of the MTD with regards to CPT structural derivatives. Among them, cyclodextrin conjugates as CRLX101 seem to be the best positioned to achieve regular clinical use, according to the promising efficacy and tolerability results, as well as plasma half-life values obtained in Phase II trials. However, most of these systems show limited functionalization ability to bind covalently the drug, which limits the loading capacity, as well as the possibility to combine CPT with other therapeutic molecules and/or imaging agents. In this sense, next generation of CPT delivery vehicles should merge properties of organic and inorganic materials. Whilst organic components will provide good stability in physiological fluids, low toxicity and immunogenicity, and minimized reactions with serum proteins, inorganic materials (which alone could present strong limitations for biological use, due to possible compositional toxicity and severe interaction with serum proteins), may offer almost unlimited external surface area to bind different active molecules and stimuli-sensitive linkers and devices. Obviously, from the regulatory point of view it is much more challenging to develop hybrid nanocarriers, which require the evaluation of every single component and, possibly, their clinical translation will not be as smooth as that of organic polymers, but the potential of these systems points out to solving the strong interpatient variability that currently hampers the widespread use of CPT nanopreparations. In this review, we have tried to reflect some of the most interesting candidates to achieve the clinics soon.

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