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Tumor-targeting drug delivery of chemotherapeutic agents*

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Abstract: Despite the significant progress in the development of cancer detection, prevention, surgery, and therapy, there is still no common cure for this disease. In addition, the long-standing problem of chemotherapy is the lack of tumor-specific treatments. Traditional chemotherapy relies on the premise that rapidly proliferating cancer cells are more likely to be killed by a cytotoxic agent. In reality, however, cytotoxic agents have very little or no specificity, which leads to systemic toxicity, causing undesirable severe side effects. Therefore, various "molecularly targeted cancer therapies" have been developed for use in specific cancers, including tumor-targeting drug delivery systems (TTDDS). In general, a TTDDS consists of a tumor recognition moiety and a cytotoxic "warhead" connected through a "smart" linker to form a conjugate. When a multi-functionalized nanomaterial is used as the vehicle, a "Trojan horse" approach becomes possible for mass delivery of cytotoxic warheads to maximize the efficacy. This account presents the progress in the molecular approaches to the design and development of novel drug delivery systems for tumor-targeting chemotherapy in our laboratory.

Keywords: anticancer activity; biomedical applications; drug delivery; drug discovery; medicinal chemistry; nanomaterials.

INTRODUCTION

Cancer is a leading cause of death in the world, and remains one of the most challenging diseases to fight against. Traditional chemotherapy relies on the premise that rapidly proliferating tumor cells are more likely to be destroyed by cytotoxic agents than normal cells. In reality, however, these cytotoxic agents have little or no specificity, which leads to systemic toxicity causing undesirable side effects such as neutropenia, anemia, hair loss, damage to liver, kidney and bone marrow, etc. Thus, the development of tumor-specific drug delivery systems for anticancer agents, recognizing the intrinsic differences between normal and cancer cells/tissues, is an urgent need for efficacious cancer chemotherapy. Various drug delivery systems have been investigated over the past few decades to address this problem [1]. Rapidly growing cancer cells overexpress tumor-specific receptors to enhance the uptake of nutrients and vitamins. These receptors can be used as targets to deliver cytotoxic agents specifically to cancer cells through receptor-mediated endocytosis (RME). Moreover, the physiological characteristics of tumor and cancer cells can be exploited to selectively accumulate and release an anticancer agent inside them. Monoclonal antibodies (mAb), polyunsaturated fatty acids (PUFAs), folic acid, aptamers, trans-

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ferrin, oligopeptides, and hyaluronic acid, for example, have been employed as tumor-specific "guiding modules" to construct tumor-targeting drug conjugates [1–3].

Tumor-targeting drug conjugates, which can be regarded as "guided molecular missiles" against cancer, typically consist of a tumor-targeting module (TTM) connected to a cytotoxic warhead directly or through a suitable "smart" linker (Fig. 1). This conjugate should be nontoxic and stable in blood circulation to minimize systemic toxicity and should be effectively internalized inside the target tumor cells. Upon internalization, the conjugate should efficiently release the anticancer agent without loss of potency [1,4–6]. We describe here an account of our research on the design, synthesis, and biological evaluation of novel tumor-targeting drug delivery systems (TTDDS) for new-generation taxoid anticancer agents.

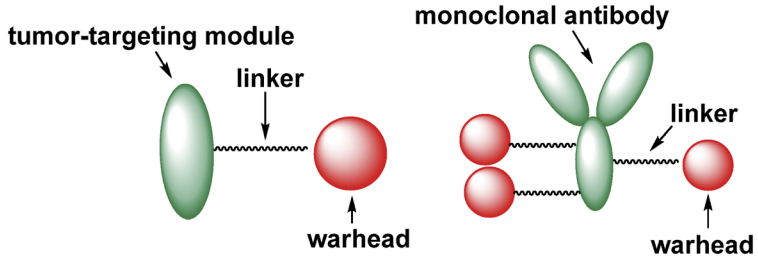


Fig. 1 Tumor-targeting drug conjugates.

SECOND-GENERATION TAXOID AS "WARHEAD"

Paclitaxel and docetaxel have brought about significant impact on the current cancer chemotherapy, mainly because of their unique mechanism of action [7], but seriously suffer from the lack of tumor specificity and multidrug resistance (MDR). Paclitaxel and docetaxel are effective against breast, ovary, and lung cancers, but do not show efficacy against colon, pancreatic, melanoma, and renal cancers. For example, human colon carcinoma is inherently multidrug-resistant due to the overexpression of P-glycoprotein (Pgp), which is an effective ATP-binding cassette (ABC) transporter, effluxing out hydrophobic anticancer agents including paclitaxel and docetaxel [8]. On the basis of our structure–activity relationship study of taxoids, we have developed a series of highly potent new-generation taxoids [9–13], including "ortataxel" which has advanced to phase II human clinical trials. Most of these taxoids exhibited 2–3 orders of magnitude higher potency than those of paclitaxel and docetaxel against drug-resistant cell lines expressing MDR phenotypes. Accordingly, these highly potent taxoids have been used as the warhead of our "guided molecular missiles". Selected new-generation taxoids are listed in Table 1 and their potencies in Table 2.

Table 1 Selected new-generation taxoids.

Taxane	R^1	R^2	R^3	X	Y
Paclitaxel	Ac	Ph	PhCO	H	H
Docetaxel	H	Ph	*t*-Boc	H	H
SB-T-1213	EtCO	*i*-butenyl	*t*-Boc	H	H
SB-T-1214	*c*-PrCO	*i*-butenyl	*t*-Boc	H	H
SB-T-1216	Me_2NCO	*i*-butenyl	*t*-Boc	H	H
SB-T-11033	EtCO	*i*-Bu	*t*-Boc	MeO	H
SB-T-121303	EtCO	*i*-butenyl	*t*-Boc	MeO	H
SB-T-121313	EtCO	*i*-butenyl	*t*-Boc	MeO	MeO
SB-T-121602	Me_2NCO	*i*-butenyl	*t*-Boc	Me	H

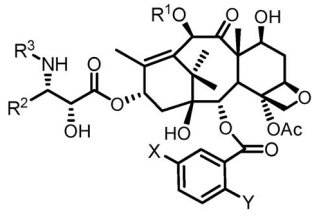


Table 2 Cytotoxicity (IC_{50}, nM) of selected new-generation taxoids against human cancer cell lines.

Taxane	MCF7[a]	NCI/ADR[b]	LCC6-MDR[c]	CFPAC-1[d]	HT-29[e]	DLD-1[f]
Paclitaxel	1.7	550	346	68	12	300
Docetaxel	1.0	723	120			
SB-T-1213	0.18	4.0		4.6	0.37	3.9
SB-T-1214	0.20	3.9		0.38	0.73	3.8
SB-T-1216	0.13	7.4		0.66	0.052	5.4
SB-T-11033	0.36	0.61	0.80			
SB-T-121303	0.36	0.79	0.90	0.89		
SB-T-121313	0.30			0.025	0.56	
SB-T-121602	0.08			0.31	0.003	0.46

[a]Human mammary cancer cell line (Pgp–).
[b]Human ovarian cancer cell line (Pgp+).
[c]*mdr1* transduced human breast cancer cell line (Pgp+).
[d]Human pancreatic cancer cell line.
[e]Human colon cancer cell line (Pgp–).
[f]Human colon cancer cell line (Pgp+).

We also investigated the activity of SB-T-1214, as representative new-generation taxoid, against colon cancer stem cells (CSCs) using cancer spheroids induced by $CD133^{high}/CD44^{high}$ cells in 3D cultures [14]. Administration of 0.1-1 μM SB-T-1214 for 48 h induced a loss of integrity of the floating spheroids and apoptosis in more than 90 % of the sphere cells. The fluorescently labeled drug revealed efficient penetration into spheroids with 30 min exposure. Most importantly, viable cells that survived this treatment regimen significantly lost the ability to form secondary spheroids, which indicates that colon CSC population was critically affected. Thus, 1000 of untreated HCT116, HT-29, and DLD-1 primary spheroid cells induced 125 ± 6, 75 ± 7, and 93 ± 6 secondary spheroids, respectively, whereas the SB-T-1214-treated dissociated spheroid cells produced only 1.5 ± 0.3, 4 ± 0.6, and 3 ± 0.4 secondary spheroids, respectively ($P < 0.01$) (Fig. 2). After placement on type I collagen surfaces, cells that survived drug treatment, displayed profound morphological abnormalities, indicating a clear sign of the mitotic catastrophe.

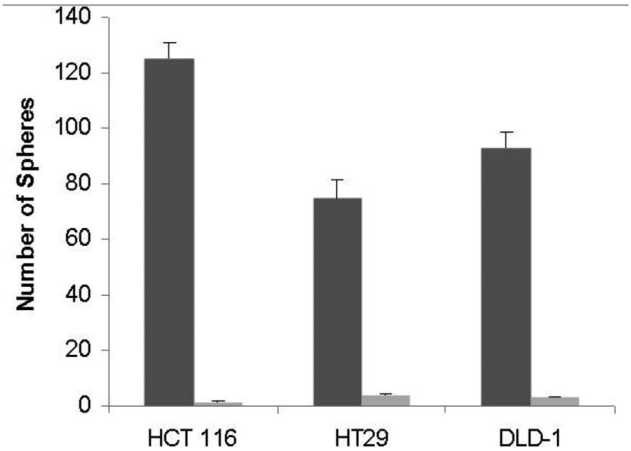


Fig. 2 Effects of SB-T-1214 on colon CSCs: Untreated cells (black) and SB-T-1214-treated cells (gray) (adapted from ref. [14]).

The CD133^{high}/CD44^{high} cell populations derived from the three analyzed colon cancer cell lines (i.e., HCT116, DLD-1, and HT29) were characterized by means of the stem cell pathway-specific PCR array assay [14]. Each array contains SYBR green-based real-time PCR gene-specific assays for a set of 84 genes. Using filtering criteria of a 1.5 or greater fold-change in expression, we have analyzed differentially expressed genes in these three types of floating colonospheres as compared to their bulk differentiated adherent counterparts, as well as before and after treatment with SB-T-1214. About one-fourth of the analyzed stem cell-related genes, including Wnt and Notch pathway genes responsible for self-renewal and cell cycle regulation, were commonly up-regulated in all types of spheroids (Fig. 3, left panel). As Fig. 3 shows, relatively low concentrations of SB-T-1214 (100 nM to 1 μM for 24 or 48 h) induced dramatic down-regulation of stemness in the majority of stem cell-related genes in all three types of colonospheres (Fig. 3, right panel).

These results provided additional strong support for the use of new-generation taxoids as the warheads of novel tumor-targeting drug conjugates.

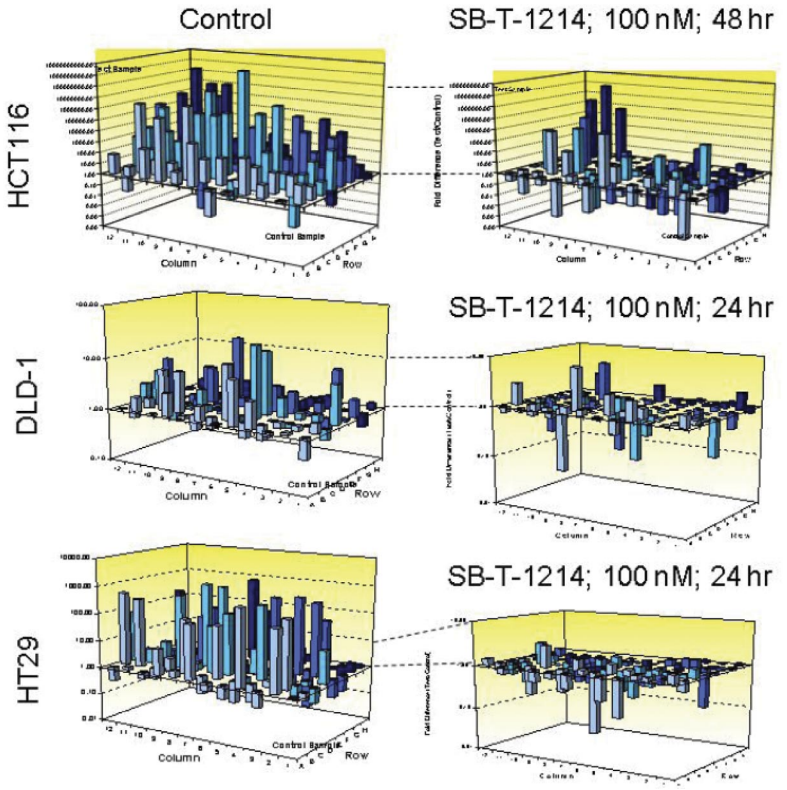


Fig. 3 Suppression of the stem cell-related gene expressions induced by SB-T-1214 (adapted from ref. [14]).

OMEGA-3 POLYUNSATURATED FATTY ACID CONJUGATES

Omega-3 PUFAs such as linolenic acid (LNA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) are naturally occurring compounds found in vegetable oils, cold-water fish, and meat. DHA is a nutritional additive approved by the FDA in the United States and is considered safe for humans [15,16]. Perfusion studies have shown that some PUFAs are taken up more rapidly by tumor cells than normal cells. In addition, some omega-3 PUFAs have shown anticancer activity against various cancer

cell lines in both clinical and preclinical trials. It has also been shown that PUFAs are readily incorporated into the lipid bilayer of tumor cells which disrupts the morphology of the cell and presumably influences the susceptibility of the tumor cells to anticancer agents [17]. DHA-paclitaxel (Taxoprexin®), which is currently in phase III clinical trials, was shown to have better stability and efficacy than paclitaxel in some studies but would not be effective against MDR tumors that overexpress Pgp [18]. As mentioned above, new-generation taxoids exhibit 2–3 orders of magnitude better activity than paclitaxel against MDR cancer cell lines. Thus, PUFA conjugates which bear a new-generation taxoid should be more efficacious than DHA-paclitaxel against drug-resistant tumors [5,19].

Novel PUFA-taxoid conjugates were synthesized and assayed in vivo for their efficacy against different drug-resistant and drug-sensitive human tumor xenografts in severe combined immune deficiency (SCID) mice. Several of these conjugates led to a complete regression of the tumor in all surviving mice with minimal systemic toxicity. For example, DHA-SB-T-1214 led to a complete regression of the highly drug-resistant DLD-1 colon tumor xenograft in 5 of 5 mice without appreciable systemic toxicity, wherein no recurrence of tumor growth was observed for more than 190 days after treatment (Fig. 4). DHA-SB-T-1213 and DHA-SB-T-1216 delayed the tumor growth of A121 ovarian tumor xenografts for more than 186 days and caused complete regression in all surviving mice [19]. The excellent efficacy of the PUFA–taxoid conjugates against drug-resistant and drug-sensitive human tumor xenografts provides bright prospect in cancer chemotherapy and warrants further preclinical and clinical development of these conjugates.

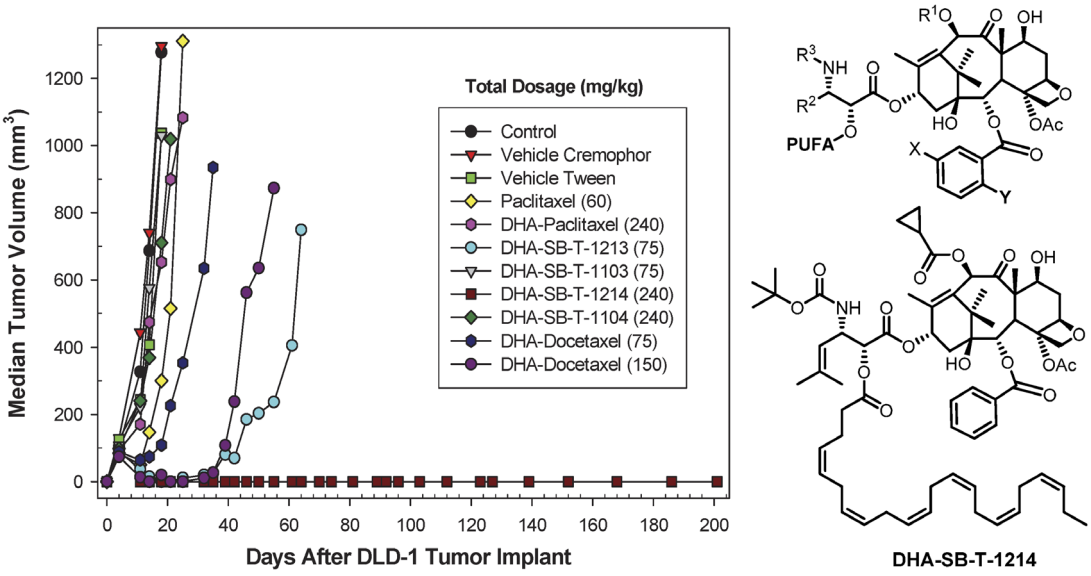


Fig. 4 Effect of DHA–taxoid conjugates on human colon tumor xenograft DLD-1.

MONOCLONAL ANTIBODY CONJUGATES

Cancer cells overexpress certain antigens on the cell surface, and these tumor-specific antigens can be used as a biomarker to differentiate tumor tissues from normal tissues [1,20,21]. Certain monoclonal antibodies (mAb) have high binding specificity to tumor-specific antigens and can be used as drug delivery vehicles to carry a payload of cytotoxic agents specifically to the tumor site. The mAb–drug conjugate is internalized upon binding to the tumor antigen via RME and the payload is released inside the cancer cell. Mylotarg® was the first mAb–drug immunoconjugate approved by the FDA in 2000 for

the treatment of acute myelogenous leukemia (AML) and several other mAb–drug conjugates are currently in clinical trials [1,20].

The efficacy of mAb–drug immunoconjugates depends not only on the specificity of the mAb and the potency of the cytotoxic drug, but also on the linker which connects the mAb to the drug. We have successfully conjugated a highly cytotoxic C-10 methyldisulfanylpropanoyl taxoid to different immunoglobin G class mAbs, recognizing the epidermal growth factor receptor (EGFR), through a disulfide-containing linker (Fig. 5) [22]. Among these conjugates, immunoconjugate KS61–SB-T-12136 exhibited remarkable tumor-specific antitumor activity in vivo against A431 squamous tumor xenografts in SCID mice, resulting in complete inhibition of tumor growth in all the treated mice with no noticeable toxicity and there was no trace of cancer cells at the end of the experiment (Fig. 6) [22]. The disulfide linker employed in this first-generation mAb–taxoid conjugates was found to be stable in

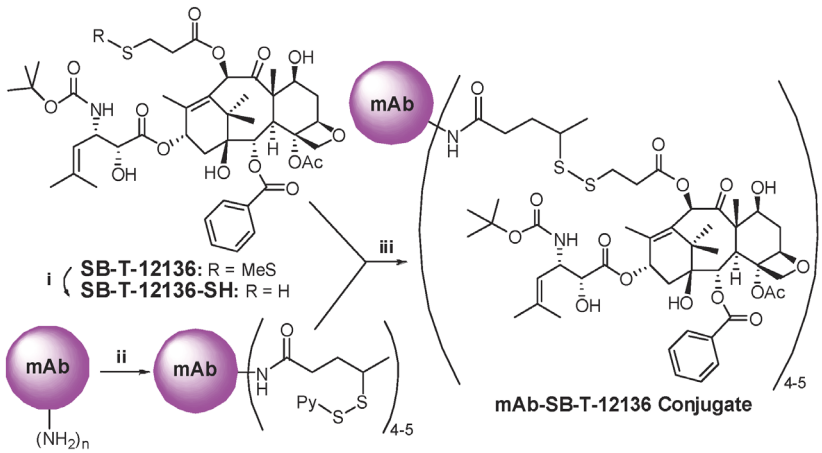


Fig. 5 Synthesis of mAb–taxoid conjugates: (i) dithiothreitol (DTT); (ii) *N*-succinimidyl-4-(2-pyridyldithio) pentanoate (**SPP**, 10 equiv in ethanol), 50 mM potassium phosphate buffer, pH 6.5, NaCl (50 mM), EDTA (2 mM), 90 min; (iii) 50 mM potassium phosphate buffer, pH 6.5, NaCl (50 mM), EDTA (2 mM), **SB-T-12136-SH** (1.7 equiv per dithiopyridyl group, in EtOH), 24 h (adapted from ref. [22]).

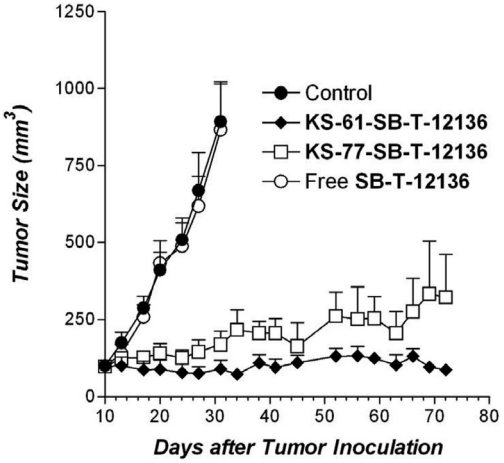


Fig. 6 Antitumor activity of anti-EGFR mAb–taxoid conjugates against A431 xenografts in SCID mice (adapted from ref. [22]).

blood plasma, but efficiently cleaved inside the tumor by glutathione or other intracellular thiols to release taxoid warhead, SB-T-12136H. However, the taxoid released through linker cleavage had a modification at C10 to introduce the disulfide linker moiety, which resulted in 8–10 times reduced potency compared to the parent taxoid [5,22]. Accordingly, mechanism-based second-generation disulfide linkers which would release unmodified parent taxoids were devised for more efficacious conjugation of taxoids to TTMs.

NOVEL SELF-IMMOLATIVE DISULFIDE LINKERS

Second-generation mechanism-based bifunctional disulfide linkers can be generally used to connect a warhead to one end and a tumor-specific guiding model to the other end. This self-immolative disulfide linker module can release a taxoid warhead efficiently inside cancer cells by taking advantage of 1000 times higher concentration of glutathione in tumor as compared to that in blood plasma [23]. When the guiding module navigates the drug-conjugate to the target receptors on the tumor surface, the whole conjugate is internalized via RME. Then, an intracellular thiol-triggered cascade drug-release takes place through thiolactonization (Fig. 7) and the released potent anticancer drug attacks its target protein, i.e., microtubules for taxoids. To promote the thiolactonization process, a phenyl moiety was strategically placed to direct the cleavage of the disulfide bond by intracellular thiol (e.g., glutathione), generating a thiophenolate or sulfhydrylphenyl species which attacks the ester linkage to the drug molecule (Fig. 7). The validity of this self-immolative drug-release mechansim has been proven in a model system using fluorine-labeling and monitoring by ^{19}F NMR spectroscopy [4] as well as in a real system with cancer cells using fluorescence-labeling and confocal fluorescence microscopy (CFM). These self-immolative disulfide linkers have been successfully incorporated to various tumor-targeting drug conjugates and their efficacy evaluated in cancer cells [5,6,24].

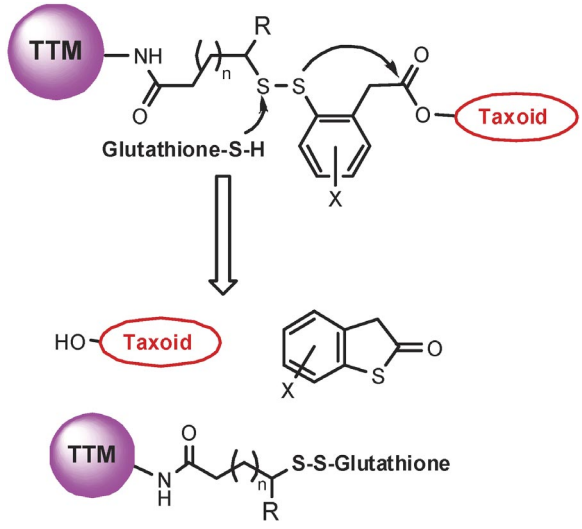


Fig. 7 Second-generation self-immolative disulfide linkers (TTM = tumor-targeting module) (adapted from ref. [5]).

VITAMINS AS TUMOR-TARGETING MODULES

Biotin and folic acid are essential vitamins involved in fatty acid metabolism and nucleotide synthesis, respectively. Cancer cells need these vitamins to maintain their rapid proliferation and thus overexpress

these vitamin receptors on the cancer cell surface [25,26]. The strategic incorporation of either biotin or folic acid into a drug-conjugate, bearing a self-immolative disulfide linker coupled with a potent taxoid, ensures tumor-targeting delivery of the drug-conjugate and internalization via RME.

Three fluorescence probes **1–3** were designed and synthesized to study the internalization mechanism, drug release, and drug binding to the target protein by means of CFM (Fig. 8) [5,6]. It was confirmed that the internalization mechanism of the drug-conjugates **1** and **3** in L1210FR (leukemia) cells was RME based on its clear temperature dependence and its almost complete blockage by the pretreatment of the cells with excess biotin. The drug release mechanism was confirmed by using biotin-linker-coumarin conjugate (**2**), which was a fluorogenic probe, hence the observation of fluorescence provides evidence for the self-immolation of disulfide linker and release of free coumarin, which is the model for a taxoid. The drug release from biotin-linker-SB-T-1214 conjugate (**3**) was further confirmed by the observation of the binding of a fluorescently labeled taxoid to microtubules. Because of a short incubation time for CFM analysis, glutathione ester was added to accelerate the cleavage of disulfide linkage in this experiment, which in turn confirmed the glutathione-triggered drug release.

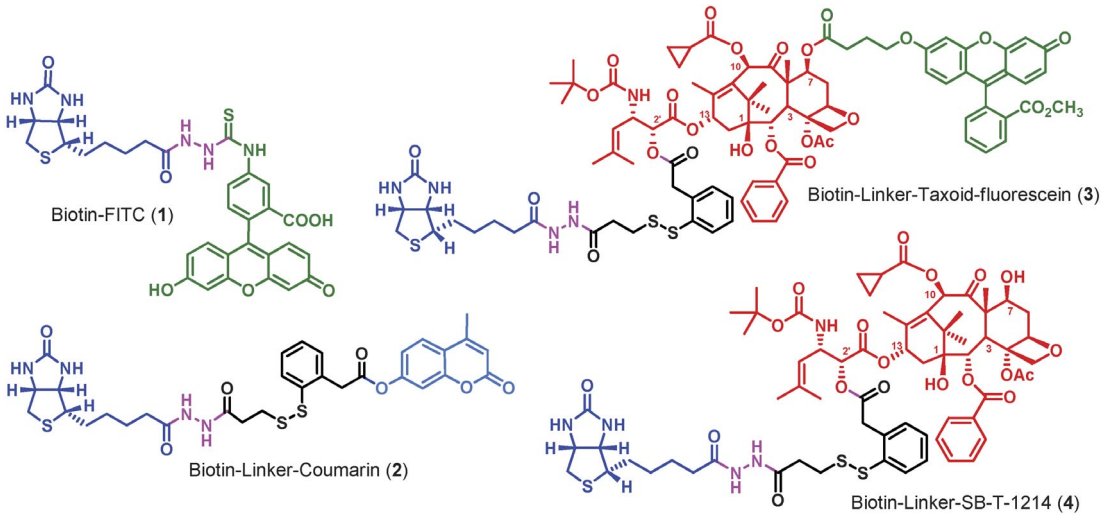


Fig. 8 Fluorescent probes (**1–3**) for RME, drug release and target protein binding.

In addition, biotin-linker-SB-T-1214 conjugate (**4**) was synthesized and assayed in vitro against L1210 (mouse lymphocytic leukemia), L1210FR (folate and biotin receptors overexpressed L1210 leukemia), and WI38 (normal human lung fibroblastoma) cells to examine the efficacy of the biomarker-specific targeting of the conjugate [5,6]. The IC_{50} values of the conjugate against L1210FR, L1210, and WI38 cell lines were 8.80, 522, and 570 nM, respectively. The results clearly indicate the high biomarker-specificity of the drug-conjugate, which is consistent with the RME-based internalization and drug release observed by CFM and flow cytometry using fluorescent probes (Fig. 9).

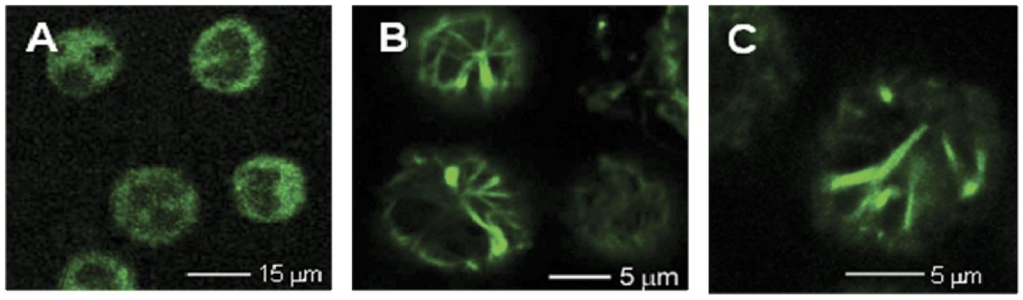


Fig. 9 (A) CFM image of L1210FR cells after incubation with the probe **3**. (B) CFM image of L1210FR cells that were initially incubated with the probe **3**, followed by treatment with GSH-OEt to release the fluorescently labeled taxoid (SB-T-1214-fluorescein). Then, the microtubule network in the cells was fluorescently labeled by SB-T-1214-fluorescein and visualized. (C) CFM image of 1210FR cells after incubation with SB-T-1214-fluorescein as a control experiment (adapted from ref. [6]).

Taxoid conjugate with dual targeting modules and that with dual warheads

As an extension of the tumor-targeting drug conjugates bearing a vitamin as the TTM, self-immolative disulfide linker and a taxoid as the warhead described above, we have designed and successfully synthesized a novel drug conjugate bearing dual targeting modules, i.e., biotin and folic acid, a trisubstituted 1,3,5-triazine splitter, a self-immolative disulfide linker and SB-T-1214 (**5**), as well as another novel drug conjugate bearing dual warheads, i.e., SB-T-1214 and topotecan, with self-immolative disulfide linkers, a biotin or folic acid as the TTM and a trisubstituted 1,3,5-triazine splitter (**6**) (Fig. 10). Their biological evaluations will be reported elsewhere in due course.

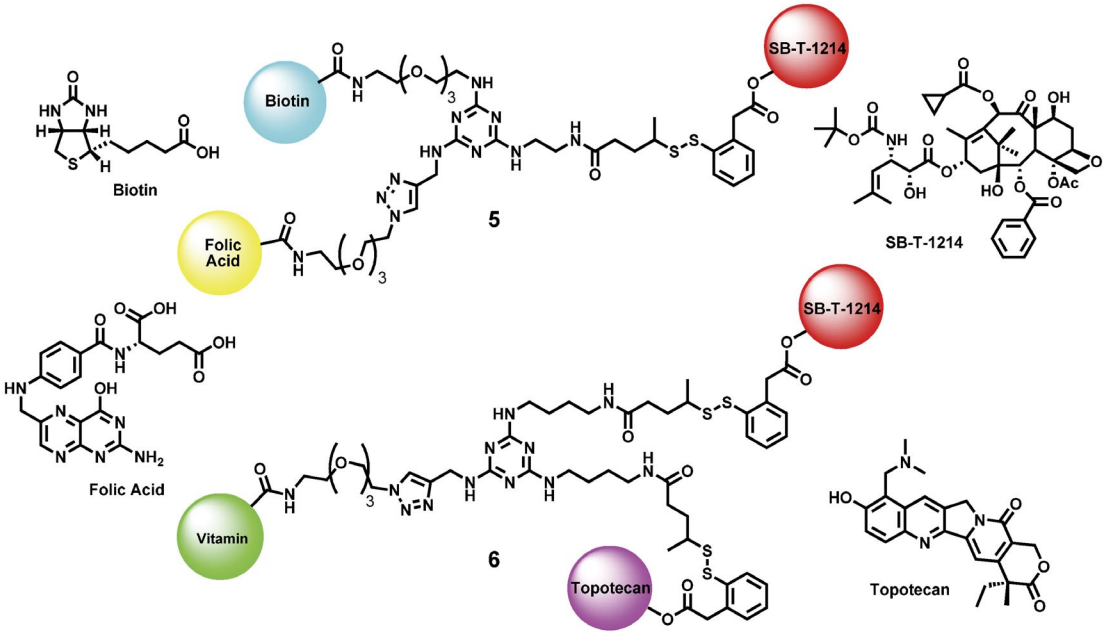


Fig. 10 Novel drug conjugates bearing dual targeting modules or dual warheads.

SINGLE-WALLED CARBON NANOTUBE AS UNIQUE VEHICLE FOR TUMOR-TARGETING DRUG DELIVERY

Single-walled carbon nanotubes (SWNTs) have been attracting substantial interest in their potential as unique drug delivery vehicles [27,28]. Functionalized SWNTs can bear multiple units of TTM and drug molecule, which can penetrate the cell membrane and accumulate in the cytoplasm, wherein the drug molecules are released [27–29]. Thus, we designed and synthesized a novel biotin-SWNT-linker-taxoid conjugate (**7**) for mass delivery of payloads to cancer cells, wherein the enhancement of internalization via RME was also expected through multivalent binding of TTM to the vitamin receptors (Fig. 11) [24]. Functionalization of SWNT begins with the oxidation of the SWNT with concentrated H_2SO_4 and HNO_3. The resulting carboxylic acid groups were converted to amines via condensation with diamines. Then, the amine termini were connected to biotin as TTM. The side wall of the SWNT was functionalized through 1,3-dipolar cycloaddition of azomethine generated in situ, bearing an amine group. These amines were conjugated to the linker-taxoid units [24].

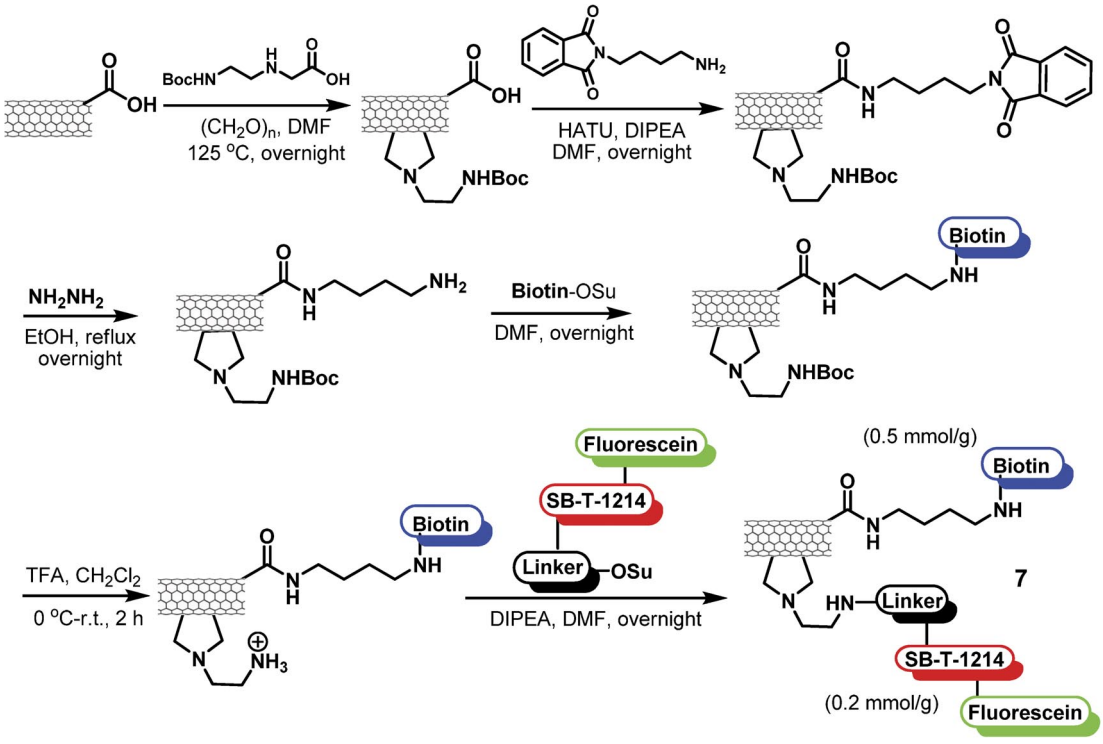


Fig. 11 Synthesis of biotin-SWNT-linker-SB-T-1214(fluorescein) conjugate (**7**) (there are multiple units of the biotin and linker-taxoid(fluorescein) tethers attached to a SWNT vehicle).

The internalization via RME, drug release, and binding to the target protein (i.e., microtubules) of fluorescein-labeled biotin-SWNT-taxoid conjugate (**7**) were investigated using CFM (Fig. 12). The results were fully consistent with those for the biotin-linker-taxoid(fluorescein) conjugate (**3**) mentioned above. The cytotoxicity assay of the conjugate **7** against L1210FR, L1210, and WI38 cell lines (IC_{50} 0.36, >50, and >50 μg/mL, respectively) has revealed excellent biomarker-specificity and substantially enhanced potency attributed to mass delivery of the taxoid warheads inside the cancer cells [24], assuring the merit of the "Trojan horse" strategy in tumor-targeting drug delivery.

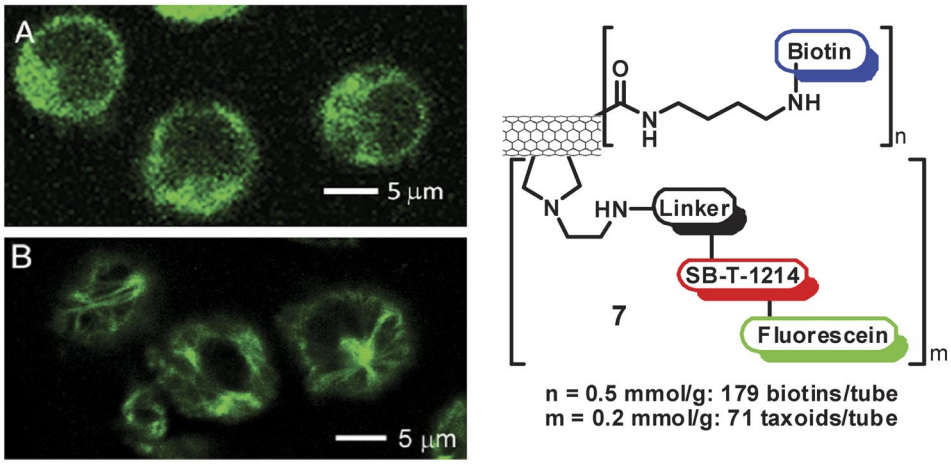
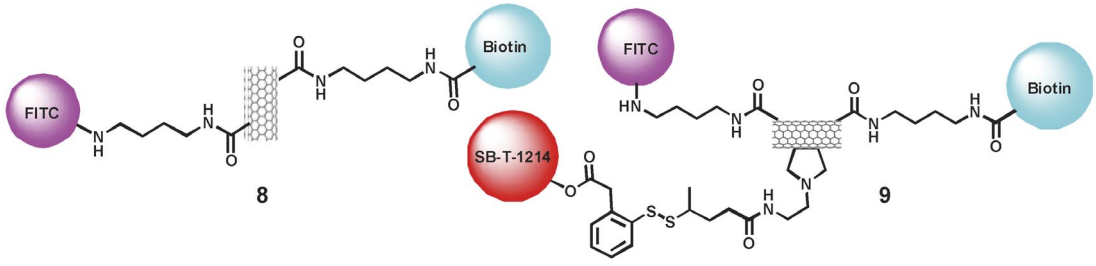


Fig. 12 Novel "Trojan horse" guided molecular missile and the CFM images of L1210FR cells treated with biotin-SWNT-linker-taxoid(fluorescein) (**7**) incubated (A) before and (B) after the addition of GSH-ethyl ester. Image B clearly highlights the presence of fluorescent microtubule networks in the living cells generated by the binding of SB-T-1214-fluorescein upon cleavage of the disulfide bond in the linker by either GSH or GSH-ethyl ester.

Thermal ablation by near-infrared light with and without cytotoxic warhead

Nanomaterials often possess unique photophysical properties, such as photothermal and photoacoustic properties, which can be explored to destroy cancer cells [30,31]. SWNTs are suitable for this approach because of their strong optical absorbance in the near infra-red (NIR) region (700–1100 nm) [32]. Carbon nanotubes (CNTs) have been explored as photothermal agents to kill cancer cells by heating the CNTs via continuous laser irradiation at high power density (3.5–35 W/cm^2) for 3–4 min [31,33,34]. Biological systems are transparent to NIR light, and the strong optical absorbance of SWNTs in this spectral window can be effectively used for the optical stimulation of the SWNTs inside the cancer cells to cause irreversible damage, leading to cell death. When SWNTs generate heat upon laser or other forms of irradiation, it causes hyperthermia in cancer cells. Hyperthermia has been used clinically for solid tumor management in combination with other forms of therapy.

We designed and synthesized two biotin-SWNT conjugates **8** and **9** to investigate their vitamin receptor-specific internalization and effects on cancer cells through thermal ablation (for **8**) as well as a combination of thermal ablation and cytotoxic effect (for **9**) [35].



First, the vitamin receptor-specific internalization of biotin-SWNT-FITC conjugate (**8**) was confirmed by exposing L1210 cells, overexpressing biotin receptors (br+), and L1210 cells without biotin receptors overexpressed (br–) at 37 °C for 3 h. As Fig. 13 clearly shows, conjugate **8** was internalized into L1210FR (br+) cells efficiently via RME (A), while no appreciable fluorescence was observed in

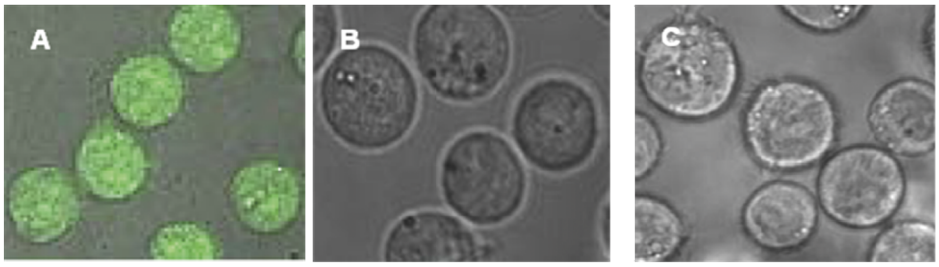


Fig. 13 CFM images of the control experiments for thermal ablation with biotin-SWNT-FITC conjugates. (A) L1210FR cells (br+) with conjugate **8** at 37 °C for 3 h. (B) L1210 cells (br-) with conjugate **8** at 37 °C for 3 h. (C) L1210 cells with NIR laser irradiation 37 °C for 7 min.

L1210 (br–) cells (B). Another control experiment was carried out to see possible effects of NIR laser irradiation on L1210FR cells. As Fig. 13C unambiguously indicates, there was no damaging effect of such irradiation on the cells.

Next, L1210FR cells with internalized conjugates **8** and **9** were irradiated by a NIR laser light at 37 °C for 5 min. As Fig. 14A shows, a clear morphological change by thermal ablation was observed on some L1210FR cells, which were exposed to conjugate **8** and NIR irradiation. When the same leukemia cells were exposed to conjugate **9** bearing cytotoxic warhead (SB-T-1214) and NIR irradiation, a dramatic morphological change was observed in some cells, indicating synergistic effects of thermal ablation and cytotoxic agent on the effective destruction of cancer cells. These results further signify the advantage in using SWNT-based TTDDS.

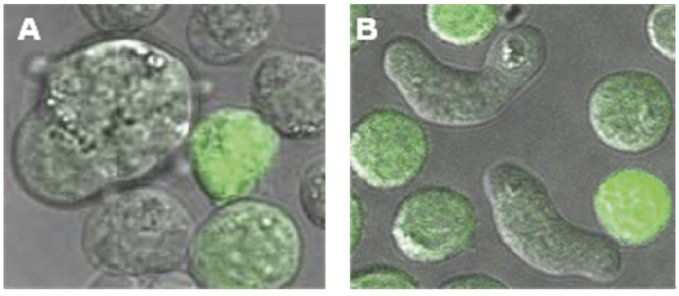


Fig. 14 CFM images of L1210FR cells exposed to biotin-SWNT-FITC conjugates and thermal ablation by NIR laser irradiation. (A) with conjugate **8** and NIR irradiation at 37 °C for 5 min. (B) with conjugate **9** and NIR irradiation at 37 °C for 5 min.

CONCLUDING REMARKS

Tumor-targeting drug conjugates have been successfully designed and constructed. These novel drug conjugates consist of TTMs (PUFAs, mAb's, and biotin), mechanism-based self-immolative disulfide linkers and warheads (new-generation taxoids). The PUFA-taxoid conjugates and the mAb-taxoid conjugates have exhibited remarkable efficacy against human tumor xenografts in animal models with minimal systemic toxicity. CFM studies using fluorescent and fluorogenic probes have unambiguously confirmed the designed internalization of drug-conjugates via RME and drug release via glutathione-triggered self-immolation of the disulfide linker. The use of multifunctionalized SWNTs as a drug delivery system bearing multiple warheads and multiple targeting modules has shown highly promising results on the benefit of mass delivery ("Trojan horse" strategy) of anticancer drug molecules

to cancer cells with high specificity. Another advantage of using SWNTs as the vehicle has been demonstrated for their ability to induce thermal ablation upon irradiation with NIR laser light. A dramatic synergism was observed by combining thermal ablation and cytotoxic warheads in an SWNT-based TTDDS, which would provide potentially a powerful method for cancer chemotherapy. Our future efforts will be concentrated on the design and synthesis of "tailor-made nano-medicines", consisting of a well-defined vehicle, multiple targeting modules (including dual targeting molecules), self-immolative smart linkers, highly potent cytotoxic warheads, and imaging modules (for MRI, PET, and fluorescence imaging), which would enable us to perform diagnostics and therapy in real time.

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