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Current drug research on PEGylation with small molecular agents

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ABSTRACT

PEGylation, covalent attaching polyethylene glycol (PEG) polymers to therapeutic agents, is one of the most promising techniques to improve the therapeutic effect of drugs. Initially, this technology is mainly applied with macromolecular drugs, such as proteins, enzymes, with ten PEGylated biomacromolecules approved by the FDA for the treatment of related diseases. The clinical successful use of PEGylated macromolecular drug has promoted the application of this technology with small molecules drugs to overcome shortcomings associated with therapy, such as possible low solubility, high toxicity, undesirable pharmaceutical characteristics and nonspecific biodistribution profiles. So far, four PEGylated small drugs have been taken into clinical trials. This review mainly focuses on the recent advances of PEGylated small molecules, including their general configuration, and the current merits and limits of PEG modification. Herein PEG delivery systems are distinguished by therapeutic application (anti-tumor, anti-inflammatory, *etc.*) and their corresponding PEGylated small molecules are described in detail.

Contents

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1. Introduction

Poly(ethylene glycol), PEG, is a synthetic polymer comprised of repeating ethylene oxide subunits with molecular weight (MW) of 44 Da (Fig. 1) [1]. Being nontoxic, non-immunogenic, non-antigenic and amphiphilic, PEG has been approved by FDA for human oral, intravenous, and dermal pharmaceutical applications [2]. Thanks to these favorable properties, PEG as modifying polymer now plays an important role in drug delivery, a strategy termed PEGylation.

Initially, PEGylation was generally described as the modification of proteins, peptides or small organic molecules by covalent binding with one or more polyethylene glycol (PEG) chains with different molecular weights [3]. PEGylation was first reported by Davies and Abuchowsky in the 1970s, for the modification of albumin and catalase [4–7]. Since then, this technology has been widely used in drug research, resulting in substantial academic advance and commercial success. PEG-drug conjugates have several advantages: prolonged residence in body, decreased degradation by metabolic enzymes, reduction or elimination of protein immunogenicity, and so on [6–9]. To date, ten PEGylated proteins, antibody fragments, and oligonucleotides been approved by FDA are on the market, including PEGylated bovine adenosine deaminase: pegademase bovine (Adagen®); and PEGylated L-asparaginase: pegaspargase (Oncaspar®); PEGylated products of interferon-α (IFN-α): peginterferon α-2b (PegIntron®) and peginterferon α-2a (Pegasys®) [1,2,10]; PEGylated granulocyte colony stimulating factor (G-CSF): pegfilgrastim (Neulasta®) [1,2,10]; PEGylated growth hormone receptor antagonist: pegvisomant (Somavert®) [4,5]; PEGylated a 28-nucleotide aptamer against vascular endothelial growth factor (VEGF); Pegaptanib sodium (Macugen®) [5,7,8]; continuous erythropoietin receptor activator Mono-mPEG-epoetin-β (Mircera®) [1,7,12]; PEGylated Fab′ fragment of the humanized anti-tumor necrosis factor (TNF)-α monoclonal antibody certolizumab pegol (Cimzia®); and PEGylated recombinant porcine uricase (urate oxidase) (Puricase®) [10–12]. Some details of these drugs are shown in Table 1 [13].

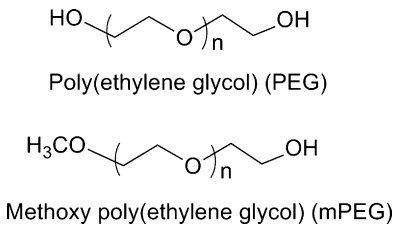


Fig. 1. Structure of PEG and mPEG.

At present, PEGylation technologies are widely used in drug modification, with an ever-increasing range in proteins, peptides, oligonucleotides and small organic molecules. Several excellent reviews have been published on description of the PEGylation of biomacromolecules [1–3,5–9,15,16], but few or no specialized reviews on PEGylated small molecular drugs have been reported in the literature. In this review we will mainly focus on the PEG modification of small molecular agents, and discuss the approaches and the utilization of modern PEGylation concepts in drug development.

2. PEGylation applied in small organic molecules

Small molecular drugs, especially the antitumor agents, often suffer some problems, such as low solubility, high toxicity, rapid excretion or untargeted biodistribution [16]. To overcome the obstacles, one promising approach is to use a PEGylation strategy. In recent years, although no approved product has yet reached the market, four PEGylated small organic drugs are currently undergoing clinical trials (see Table 2) [17]. NKTR-118 (PEG-naloxol), an orally administered PEGylated version of naloxol (an opioid antagonist) [12], is now under phase III clinical trials for the treatment of debilitating conditions such as opioid-induced bowel dysfunction (OBD) and opioid-induced constipation (OIC), both of which can occur

Table 1
FDA-approved PEGylated drugs.

Commercial name	Drug name	Company	PEG size (Da)	Indication	Year of approval
Adagen®	Pegadamase	Enzon	Multiple linear 5000	SCID	1990
Oncaspar®	Pegaspargase	Enzon	Multiple linear 5000	Leukemia (ALL, CML)	1994
PEG-INTRON®	Peginterferon-α2b	Schering-Plough	Linear 12,000	Hepatitis C	2000
PEGASYS®	Peginterferon-α2a	Hoffman-La Roche	Branched 40,000	Hepatitis C	2001
Neulasta®	Pegfilgrastim	Amgen	Linear 20,000	Neutropenia	2002
Somavert®	Pegvisomant	Pharmacia & Upjohn	4–6 linear 5000	Acromegaly	2003
Macugen®	Pegaptanib	Pfizer	Branched 40,000	Age-related macular degeneration	2004
Mircera®	mPEG-epoetin-β	Hoffman-La Roche	Linear 30,000	Anemia associated with chronic renal failure	2007
Cimzia®	Certolizumab pegol	UCB	Branched 40,000	Reducing signs and symptoms of Crohn's disease	2008
Puricase1®/Krystexxa®	PEG-uricase	Savient	10,000	Gout	2010

ALL: acute lymphoblastic leukemia; CML: chronic myeloid leukemia; GH: growth hormone; SCID: severe combined immunodeficiency disease.

during opioid pain management [12,18]. NKTR-102 (PEG-irinotecan), a PEGylated form of the topoisomerase I inhibitor irinotecan, is now under phase III/II clinical trials for the treatment of solid tumors [12,19]. EZN-2208 (PEG-SN38) is being developed by Enzon Pharmaceuticals under phase II for the treatment of advanced colorectal cancer, and its prodrug pegamotecan (PEG-camptothecin) was dropped by Enzon during phase II b trials in 2005 due to a lack of efficacy [12,20]. NKTR-105 (PEG-docetaxel) is a conjugate of PEGylated docetaxel that is now under phase I clinical trials for the treatment of solid tumors [8].

Compared to biological macromolecules, small organic molecules present fewer problems in the chemistry of PEGylation because of they have fewer functional groups, lower conformational constraints, and easier purification and characterization steps [12,17]. According to the characteristics of the bond between PEG and active agents, the modifications are classified as "permanent" or "releasable" PEGylation. Permanent PEG links create novel compounds for increasing oral bioavailability and decreasing penetration of specific barriers, such as NKTR-118 (permanent PEGylation of naloxol), which has entered phase III clinical trials [18]. Generally, permanent PEGylation requires low molecular-weight PEGs (Mw < 1000 Da) because macromolecular PEGs may block activity of small active agents at the target cells *via* steric hindrance. For releasable PEG attachments, namely the "prodrug approach", the conjugate must be chemically or enzymatically transformed into their active form after administration [12,15,16,21]. Releasable PEGylation requires large molecular-weight PEGs (1000–60,000 Da) to increase the circulating half-life, modify biodistribution and enhance water solubility.

With releasable PEG systems, too rapid breakdown of the conjugate can lead to spiking of the parent drug and possible toxicity, while too slow a release rate will attenuate the drug efficacy. Thus, releasable PEGylation should fulfill two major requirements: (1) prevent conjugated component from degradation during the transfer to the site of drug action and (2) release drug(s) from the conjugate inside the targeted cells. A well-designed prodrug of conjugation could possibly prevent adverse side effects to normal tissues and facilitate uptake in the targeted organ, tissues and cells [16,17,22].

3. General configuration of PEGylation with small organic molecules

In PEGylation, linear PEGs are the simplest and most often used conjugate agents. In these cases, active small molecules are conjugated to the distal ends of a PEG carrier [7,9,23]. Such "simple" PEG carrier could substantially enhance the properties of the drugs. The most obvious effect of PEGylation in this system is to increase solubility or sustained release of parent drug, which in turn increases cellular drug availability, decreases toxicity and enhances specific activity [12,13,16,24]. For example, in order to improve the pharmacokinetics properties and lower dose-related toxicity of the drug zidovudine (AZT), we designed and synthesized a sustained-release prodrug PEGylated AZT (Fig. 2), introduced detail in Section 5.2.3 [25].

Since linear PEG has only two sites available for the conjugation, only one or two drug molecules may be conjugated, which limits the loading capacity [3,11,26]. Furthermore, modification by PEG results in increased viscosity of PEGylated conjugates compared to the

Table 2
PEGylated small molecular drugs conjugates in clinical.

PEG conjugates	Trade name	Company	PEG size (kDa)	Indication	Stage of development
PEG-naloxol	NKTR-118	Nektar	Linear PEG	Opioid-induced constipation	Phase III
PEG-irinotecan	NKTR-102	Nektar	4-Arm PEG	Solid tumor	Phase III/II
PEG-SN38	EZN-2208	Enzon	4-Arm PEG	Solid tumor	Phase II
PEG-docetaxel	NKTR-105	Nektar	4-Arm PEG	Solid tumor	Phase I

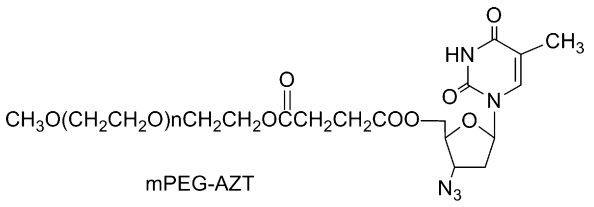


Fig. 2. The structure of mPEG-AZT.

soluble drug. In addition, some investigators claim that the comparatively large molecular weight of PEG conjugation may impede the release of the small molecular weight drugs, so that they do not reach therapeutic concentrations at the target sites [23,27,28]. To overcome these potential shortcomings, several new types of PEG have been synthesized, including branched PEG, forked PEG and multi-armed PEG [27–29]. The representatives of nonlinear PEG molecular structures mentioned above are illustrated in Fig. 3.

Branched PEG (PEG2) has an "umbrella like" structure, linking two linear mPEGs to active groups of amino acids (*e.g.*, glutamate, lysine) [21,22]. These structures give better protection than linear PEG toward approaching proteolytic enzymes, antibodies, *etc.*, attributed to the higher molecular weight and "umbrella like" structure of the branched polymer. This technology is preferred in protein PEGylation, but is not applied as frequently with small molecules [1,29,30].

Forked PEG provides multi-proximal reactive groups at the end of one or both ends of a linear PEG chain [23,30]. This structure effectively increases the drug load of the PEG by increasing active sites, but some investigators [24] point out that the maximum number of active ingredients that can be conjugated to the dendritic termini is limited mainly by the solubility of components. For example, preliminary data showed that only three molecules of camptothecin can be conjugated to such system to ensure satisfactory solubility of the whole conjugate [31].

A multi-armed PEG is a star-like structure carrying multi-hydroxyl or functional groups, increasing the active sites while simultaneously increasing the molecular weight [3,24]. This structure now is widely used in conjugation of small molecules, and several multi-armed PEG, such as NKTR-102 (PEG-irinotecan), EZN-2208 (PEG-SN38), and NKTR-105 (PEG-docetaxel), have entered into clinical trials.

4. Targeting effect of PEGylated small organic molecules

As with other polymer prodrugs, PEGylated small drugs show "passive targeting", or selective distribution pattern *in vivo* [13,15,32]. The polymer conjugation can significantly prolong the circulation time of low molecular weight therapeutic agents because the macromolecules barely permeate the endothelium of normal blood vessels. Thus, macromolecular prodrugs are more likely to be retained in tumors or other pathological tissues with disorganized vasculature [33], exhibiting enhanced permeability and retention (EPR). The passive targeting of PEGylated small drugs is described in many published papers, for example, the biodistribution of a series of PEGylated Doxorubicin (PEG-Dox) conjugates with peptide linkers [14,33,34] was evaluated in mice, with results showing that PEG-Dox conjugates had higher Dox concentrations than free Dox in tumor tissues, and also, it had low concentrations in other organs such as heart tissues, in which heart toxicity is one of the severe therapeutic limitation of free Dox.

Although the EPR effect of PEGylated macromole is efficient to drug therapy, the passive targeting still suffers several limitations, including variable vascular hyperpermeability for different tumor tissue and low cellular uptake for macromolecular prodrug [32,35]. To overcome the

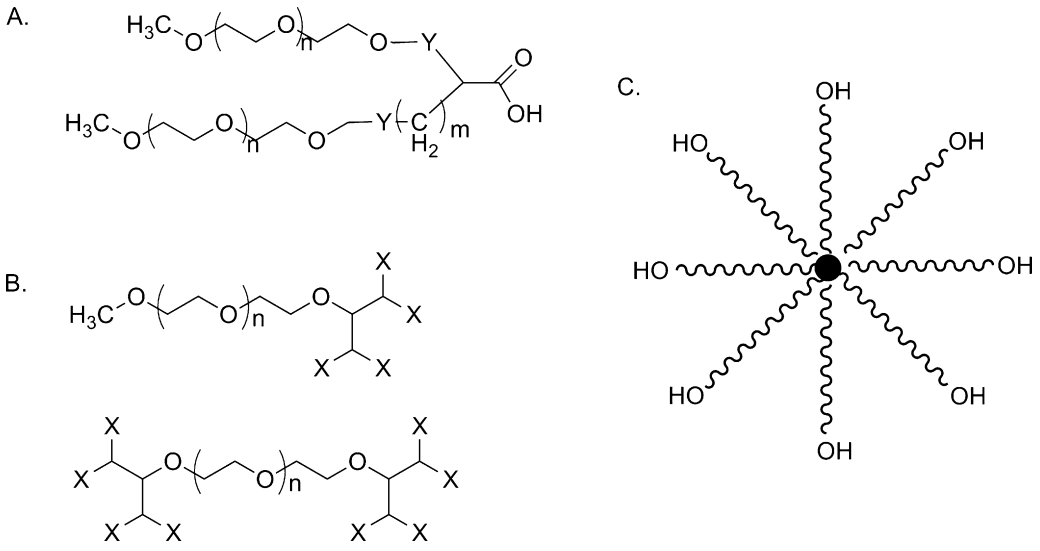


Fig. 3. Representative new types of PEG. (A) Branched PEG; two linear mPEGs are linked to amino groups of lysine, where Y represents the linker. (B) Fork-shaped PEG; X represents functional groups. (C) Multi-arm PEG; carrying multi-hydroxyl or functional groups, eight-arm PEG is prepared by hexaglycerine as a core.

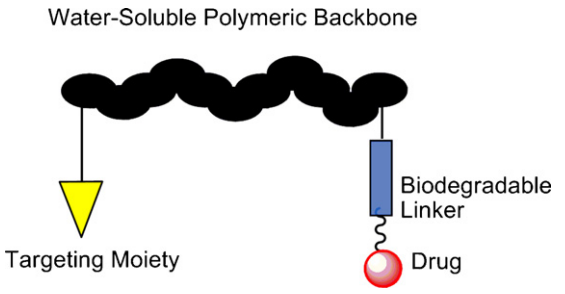


Fig. 4. Ringsdorf's model of polymer–drug conjugates.

drawbacks and enhance the therapeutic index, a model constructed by Ringsdorf in 1975 [35,36] (see in Fig. 4) was applied in polymer conjugation, consisting of a polymeric carrier, a drug, a biodegradable linker and an active targeting moiety [37]. Because pathogenic cells tend to over-express specific antigens, receptors, or transporters, various targeting moieties can be used in this model, such as antibodies, antibody fragments, peptides, aptamers, and small molecule ligands [32–35]. An active targeting system combined with PEG has became a dominant in design of drug PEGylation, and a variety of related studies have be reported in the literature, especially in the field of anticancer drug research. Jayant et al. selected sialic acid (SA) as a targeting moiety to connect with PEGylated Dox, and this targeted conjugate showed higher anti-tumor activity when compared with free drug and non-targeted conjugates [38]. A targeting moiety was added to the PEGylated structure in this model for accelerating its uptake by cancer cells and substantially enhancing its anti-tumor activity.

5. Current research of PEGylated small molecular drugs

PEG delivery systems can be classified by different parameters: the general configuration of the system (linear, branched, dendrimeric, *etc.*), the type of linkage between components (biodegradable, nonbiodegradable, *etc.*), *etc.* [24]. In the following, the current progress in design and development of the PEGylated small drugs are discussed according to their therapeutic application (anti-tumor, anti-inflammatory, *etc.*).

5.1. PEGylated anti-tumor drugs

5.1.1. PEG-doxorubicin

Doxorubicin (Dox) is one of the most widely used anticancer drugs, with high activity in different types of tumors. Although it is pharmacologically effective in cancer treatment, its clinical applications are limited by serious toxicities, especially gastrointestinal toxicity and heart failure [39]. Thus molecular modification of Dox by PEGs has drawn much attention in recent year as a means to improve potency and reduce toxicity. Veronese et al. synthesized a series of poly(ethylene glycol) PEG-Dox conjugates with different molecular weight PEG through various peptidyl linkers (Fig. 5) [40]. This study showed that all PEG conjugates were more than 10-fold less toxic than free Dox. The plasma residence time and the tumor targeting

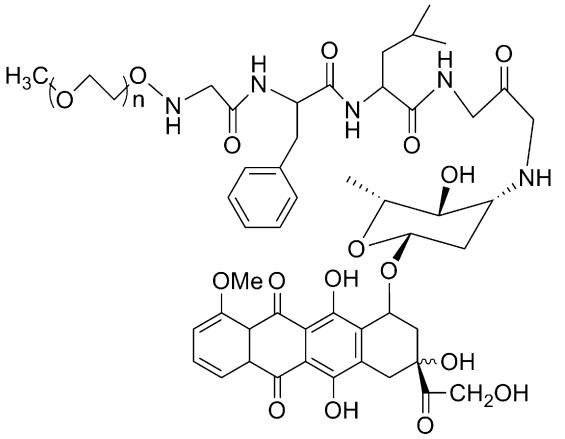


Fig. 5. A typical conjugate structure of the PEG-peptide-Dox conjugates.

concentration of PEG-Dox conjugates increased markedly compared to free Dox [40]. The use of ^{125}I-labeled PEGs showed a clear positive relationship between molecular weight and blood clearance and tumor accumulation, but there was no clear molecular weight-dependence on biodistribution.

Cao and his colleagues synthesized a novel Dox conjugate (TPGS-Dox) using D-a-tocopheryl polyethylene glycol succinate (TPGS) as the polymer carrier (Fig. 6) [41]. TPGS is a water-soluble derivative of natural vitamin E, formed by esterification of vitamin E succinate with PEG 1000. The conjugate TPGS-Dox showed nearly the same cytotoxicity and cellular uptake efficiency in comparison with the original drug Dox [41,42], but an *in vivo* pharmacokinetics evaluation, showed that TPGS-Dox resulted in 23.6 times higher AUC and 3.81 times longer half-life than the original Dox, which implied greatly enhanced therapeutic effects of the conjugate. Anbharasi et al. modified the conjugate TPGS-Dox by adding folic acid (FOL) as a target moiety (Fig. 7) [42]. *In vivo* biodistribution study indicated that TPGS-Dox-FOL exhibited a similar half-life to that with TPGS-Dox, and both of the two conjugates significantly lowered drug accumulation in the heart, thereby reducing the main side effect of cardiotoxicity. Moreover, because of active targeting of FOL, TPGS-Dox-FOL presented higher

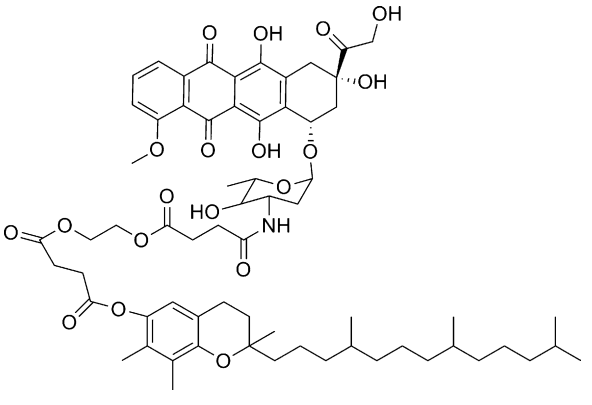


Fig. 6. Structure of TPGS-Dox.

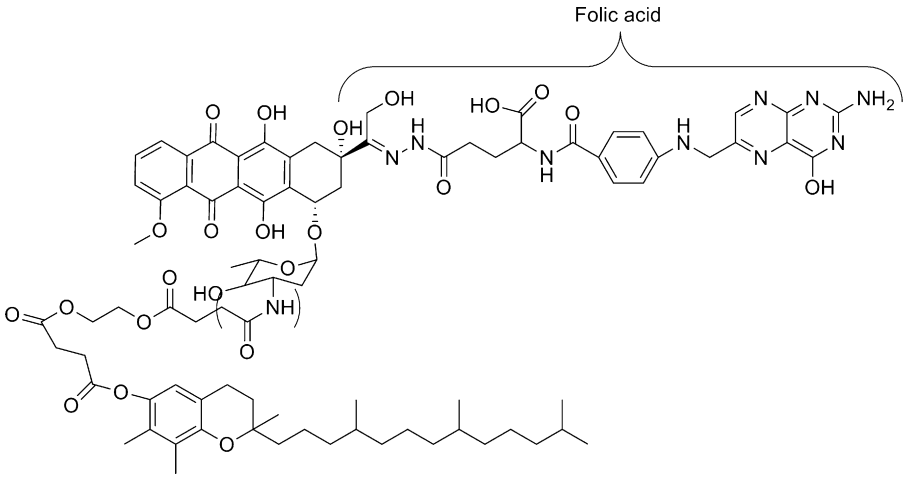


Fig. 7. Structure of TPGS-Dox-FOL.

cellular uptake of Dox than TPGS-Dox and parent Dox in tumor tissue [42].

Jayant et al. prepared a novel targeted anticancer prodrug PEG-Dox using citric acid (CA) as a spacer and sialic acid (SA) as a cancer-specific targeting moiety (Fig. 8) [38]. More than 40% of the drug was released from its conjugate in the presence of esterase enzyme, whereas the conjugate was stable at pH 7.4 in the absence of enzyme. Biological evaluation of synthesized conjugates showed that targeted prodrugs containing two copies of targeting moiety possessed substantially higher cytotoxicity when compared with free drug and non-targeted conjugates.

Jiang, Pei and coworkers prepared novel Dox conjugates using a PEGylated PAMAM dendrimer, a highly branched poly(amidoamine) with terminal amine groups, which can be linked with various biomolecules and different functionalities (*e.g.*, drugs, targeting molecules, and imaging agents) (Fig. 9) [43,44]. Dox was attached to the PAMAM dendrimers to form PPCD conjugates and PPSD conjugates *via* a pH-sensitive or pH-insensitive spacer, respectively (Fig. 10) [43]. The acid-triggered PPCD conjugates released Dox in a pH-dependent manner and showed higher cytotoxicity than PPSD against ovarian cancer (SKOV-3) cells. *In vivo* biodistribution studies with the PPCD conjugates showed that they were mainly taken up by tumor, liver and kidney, with less accumulation in the heart, spleen and lung, indicating that cardiac toxicity was significantly decreased. In further studies, Jiang et al. modified PPCD conjugates and PPSD conjugates using Arg-Gly-Asp (RGD) as an activity target moiety (Figs. 11 and 12) and evaluated their *in vitro* and *in vivo* parameters in glioma [44,45]. RGD has high affinity with integrin $\alpha_v\beta_3$, which is overexpressed in melanomas, glioblastoma, ovarian, breast, and prostate cancers. *In vitro* cytotoxicity studies showed that RGD-PPCD conjugate presented the highest cytotoxicity against C6 glioma cells compared with the other three conjugates (PPSD, PPCD and RGD-PPSD) [44]. *In vivo* distribution studies showed that the tumor accumulation of RGD-PPCD increased 1.46-fold compared to that of PPCD [45]. In addition, RGD-modified conjugates showed significantly higher cellular uptake and internalization than the RGD-unmodified ones, suggesting that RGD played a positive role in the cellular uptake of the Dox-polymer conjugates.

5.1.2. PEG-irinotecan (NKTR-102)

Irinotecan (CPT-11), a water-soluble derivative of camptothecin, inhibits the resealing of single-strand DNA breaks mediated by topoisomerase I, but it suffers some side effects, such as gastrointestinal toxicity and neutropenia

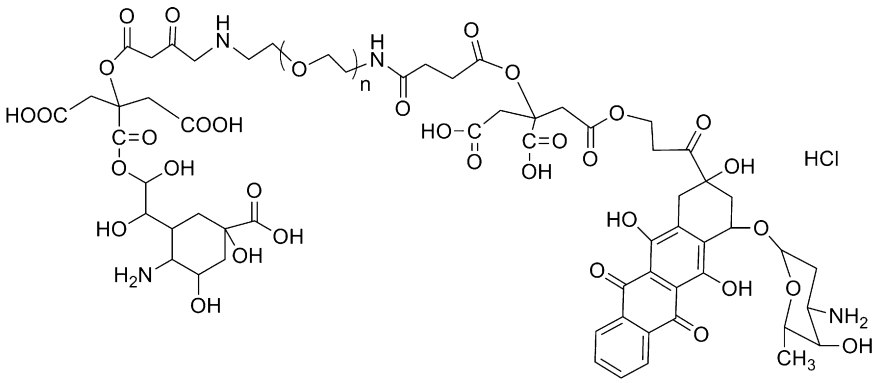


Fig. 8. Structure of SA-Dox-PEG-CA conjugate.

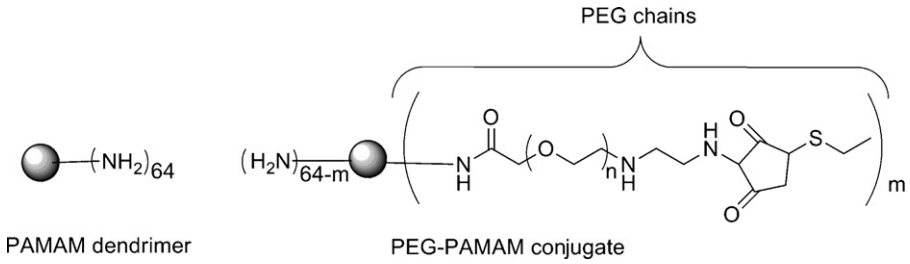


Fig. 9. Structure of PAMAM dendrimer and PEGylated PAMAM conjugate, *m* is the amount of PEG chains.

[19]. NKTR-102 (PEG-irinotecan) is a prodrug of irinotecan prepared by Nektar Therapeutics, which use a new architecture of four arms PEG as carrier [46,47] (Fig. 13). In preclinical models, accumulation of NKTR-102 in tumor achieved a 300-fold increase compared to that of the first generation topo 1 inhibitor [48–50]. The reason is due to the large molecular weight of NKTR-102, which may penetrate the leaky vasculature within the tumor tissue more readily than normal vasculature [33,34,48]. Clinical studies showed that the half-life of active drug released from NKTR-102 was greatly extended to 50 days, providing a prolonged exposure to topo I inhibition. Thereafter, 57 patients with advanced solid tumors that had failed in prior anticancer treatments or had not received available standard treatments were enrolled in a phase I clinical trial to test the safety, pharmacokinetics and antitumor activity of NKTR-102 [46]. The results showed NKTR-102 had an encouraging level of activity in a broad spectrum of tumors. At the weekly schedule (w×3 q4w, complete), the maximum tolerated dose (MTD)/recommended phase II dose (RP2D) of 115 mg/m^2, and the toxicity was manageable (diarrhea and not neutropenia was dose-limiting) [36,47]. As of this writing, NKTR-102 is in phase III clinical study for patients with metastatic breast cancer and phase II clinical study for patients with solid malignant tumors, including ovarian and colorectal cancers.

5.1.3. PEG-camptothecin

Camptothecin (CPT) is a potent anticancer agent acting through the inhibition of topoisomerase I during the S phase of the cell cycle [51,52]. However, its high toxicities and poor stability restrict clinical application [18,23]. Guiotto et al. [53] synthesized a new poly(ethylene glycol) (PEG) conjugate of 10-amino-7-ethyl camptothecin (Fig. 14). The conjugate was tested against P388 murine leukemia cell lines and proved equipotent to CPT-11, a camptothecin analog already in clinical use.

In the same year, camptothecin (CPT) was conjugated to poly(ethylene glycol) (PEG) of molecular weight 3400 using different linkers by Fleming et al. (Fig. 15) [54]. The synthetic conjugates were then loaded into biodegradable polymeric controlled-release implants, and their release characteristics were studied *in vitro*. These new conjugates showed very good water-solubility and a pH-dependent drug release. The authors implanted similar polymeric disks into rat brains and determined the concentration profile of CPT and conjugated CPT in the brain after 1, 7, 14, and 28

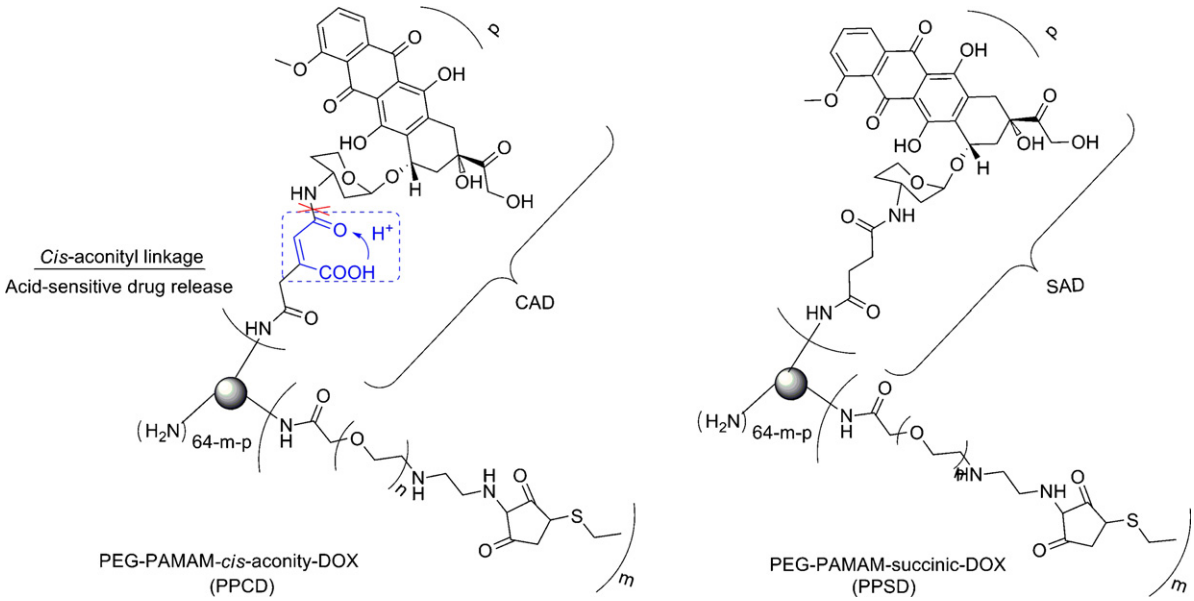


Fig. 10. Structure of pH-sensitive conjugates PPCD and pH-insensitive conjugates PPSD.

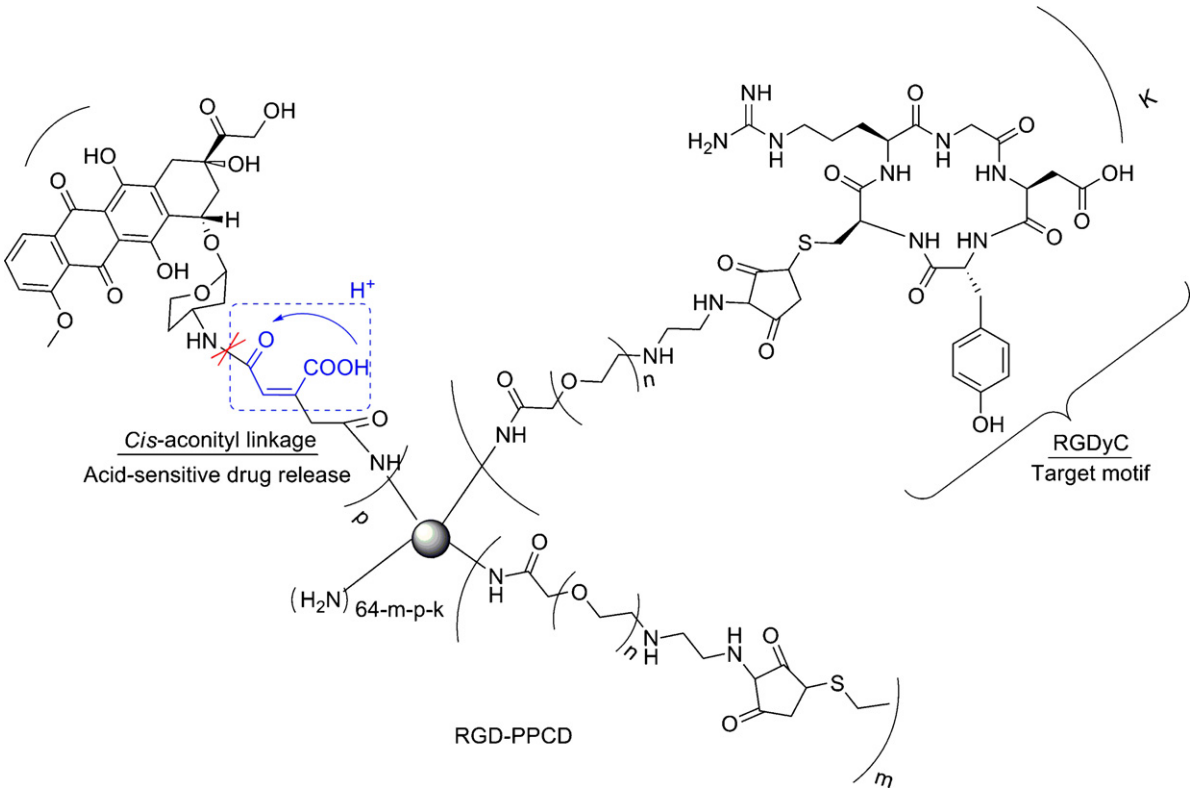


Fig. 11. Structure of PPCD with target moiety RGD.

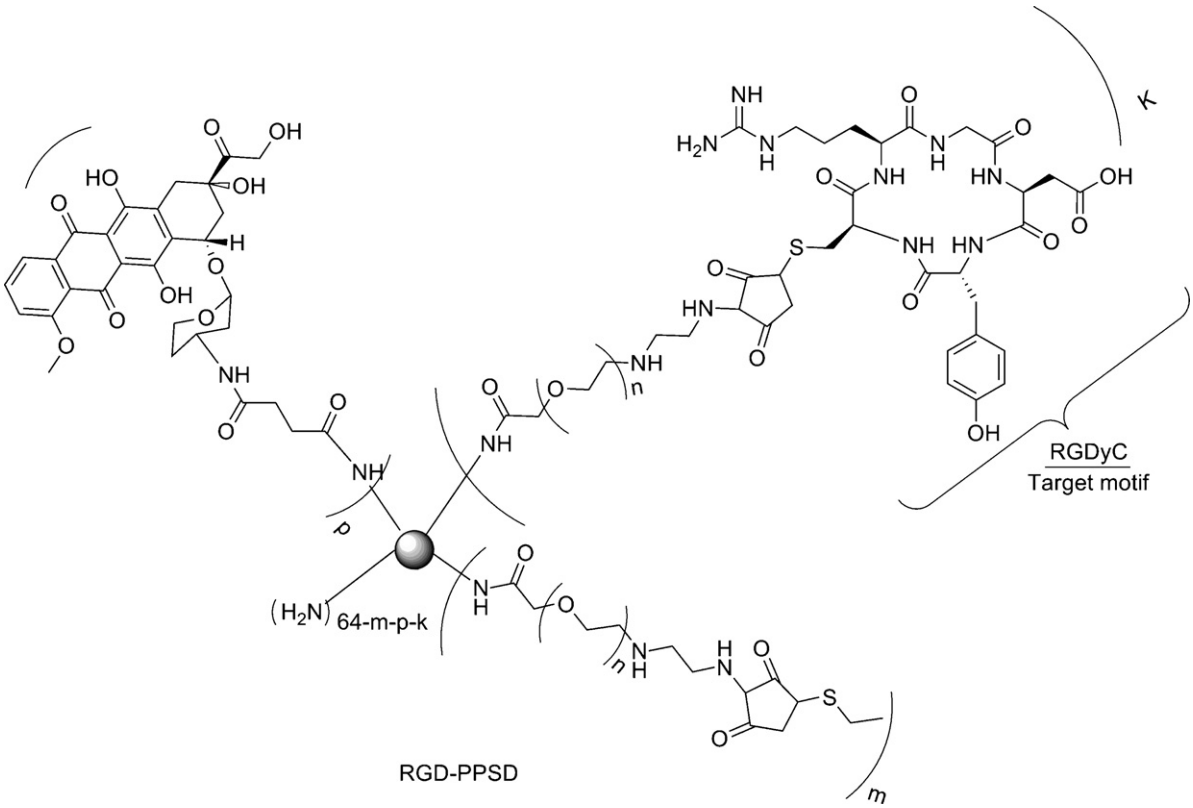


Fig. 12. Structure of PPSD with target moiety RGD.

days [54]. The results showed that PEGylation greatly increased the maximum achievable drug concentration and greatly enhanced the distribution properties of CPT. PEGylated CPT shows great promise toward increasing drug distribution after direct, local delivery in the brain.

5.1.3.1. Pegamotecan (PEGylated-camptothecin). Pegamotecan was developed from camptothecin by Enzon Pharmaceuticals, Inc. to increase the drug half-life in blood and stabilize the active lactone configuration of camptothecin by acylation. This prodrug was obtained by coupling two molecules of camptothecin to a diol PEG of 40 kDa (Fig. 16) [55–57]. A phase II study showed the conjugate appeared better tolerated, with a lower incidence of toxicities compared to irinotecan. However, the very quick *in vivo* hydrolysis of the ester between camptothecin and PEG causes the toxicity of the conjugate to be similar to that of the native drug, and the drug loading was only 1.7 wt%. Finally, the company announced that it discontinued further development of pegamotecan [12,55].

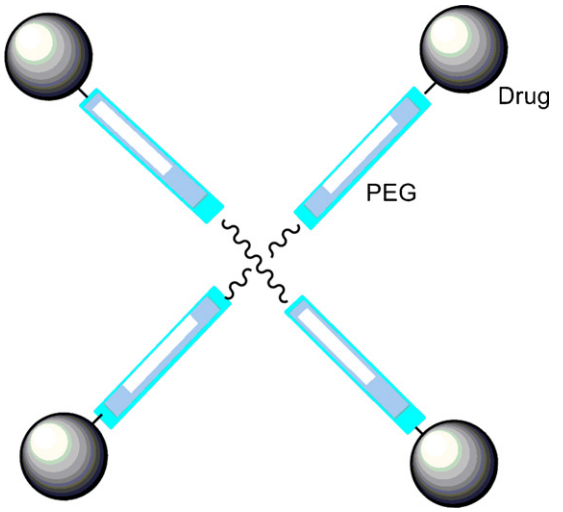


Fig. 13. Model of four arms PEG conjugated with irinotecan (NKTR-102).

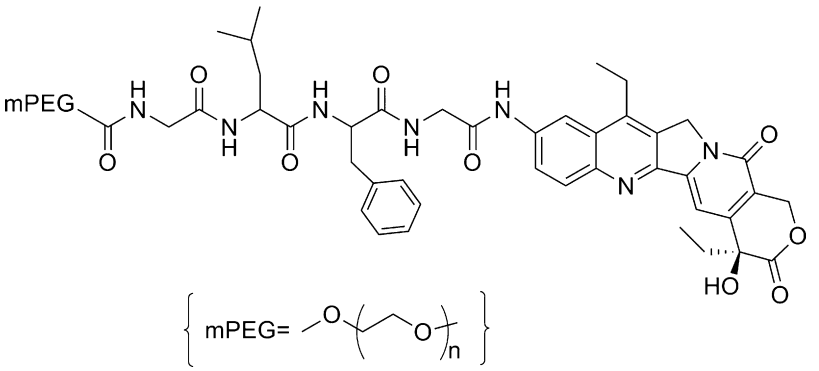


Fig. 14. Structure of poly(ethylene glycol) (PEG) conjugate of 10-amino-7-ethyl camptothecin.

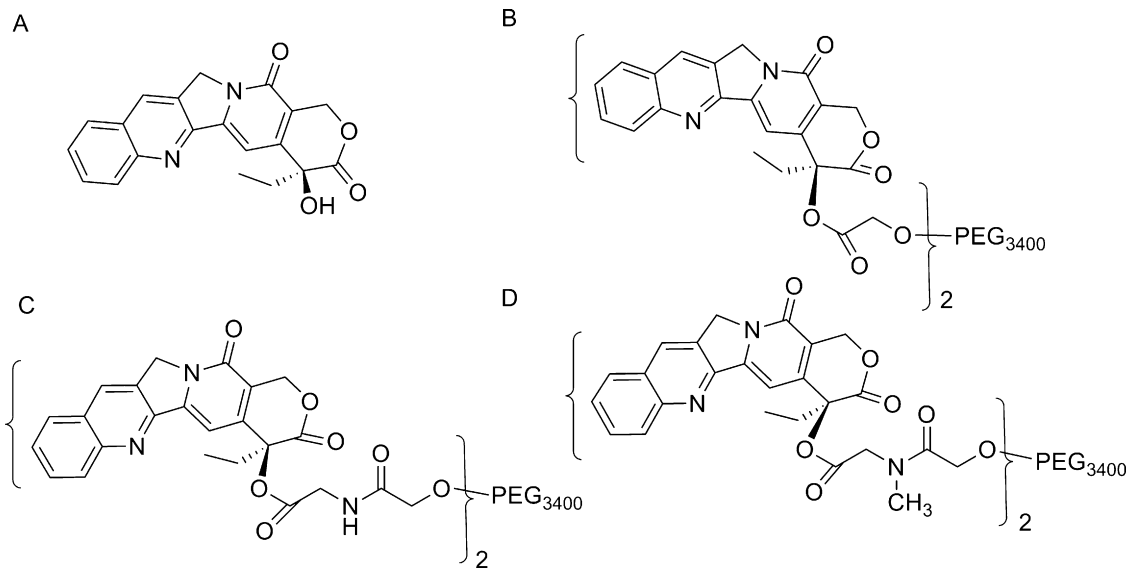


Fig. 15. Structure of PEGylated camptothecin (CPT).

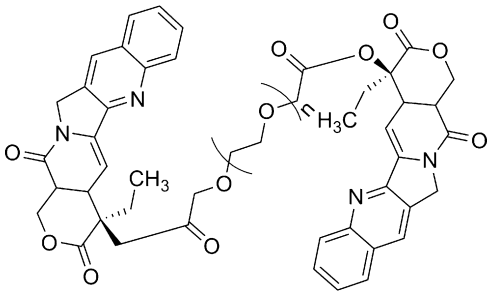


Fig. 16. Pegamotecan: camptothecin PEG 40 kDa-camptothecin.

5.1.4. PEG-SN38 (EZN-2208)

SN38 (7-ethyl-10-hydroxy-camptothecin), an active metabolite of CPT-11, has serious cytotoxicity in cell culture compared with CPT-11. Even worse, parental SN38 administration is not available because of its inherently poor water solubility [52]. Zhao et al. [58] prepared a multi-arm poly(ethylene glycol) (PEG) linked to four SN38 molecules (PEG-SN38, EZN-2208), which had high drug loading and good water solubility (Fig. 17). Two independent phase I clinical trials have been finished by the National Cancer Center in Japan and the Sarah Canon Cancer Center in the USA in patients with advanced solid tumors [59,60]. The recommended dose (RD) for the phase 2 study was the same 28 mg/m^2 in both countries [59]. The PK profile in the US study was similar to that in the Japanese study. Antitumor activity was also promising. A phase II study in patients with colorectal cancers is now underway in Japan. In the USA, two phase II studies are underway in patients with triple-negative breast cancer and patients with small cell lung cancer (SCLC) [61,62].

5.1.5. PEG-paclitaxel

Paclitaxel (PTX) is a potent anti-cancer drug, but its current clinical administration is limited because of hydrophobicity and serious side effects [63]. In early research at Enzon, several PEG-paclitaxel conjugates prepared with PEGs of different molecular weights were studied [64]. However, the company discontinued development of the PEG-paclitaxel derivative in phase I clinical trials [65].

A novel paclitaxel conjugate was prepared by Zhang et al. [66], in which paclitaxel is covalently connected to a monomethoxy-poly(ethylene glycol)-b-poly(lactide) (MPEG-PLA) block copolymer. The authors evaluated the antitumor activity of paclitaxel conjugate against the human liver cancer H7402 cells using the MTT method. The conjugate exhibited obvious cytotoxicity against H7402 cells, implying that paclitaxel is released from MPEG-PLA-paclitaxel without losing cytotoxicity.

Xie et al. [67] synthesized a triblock poly(lactic acid)-b-poly(ethylene glycol)-b-poly (lactic acid) (PLA-PEG-PLA)/paclitaxel (PTX) conjugate (Fig. 18) which can self-assemble into micelles in aqueous solutions, with a low critical micelle concentration. PLA-PEG-PLA/PTX micelles exhibit a spherical structure with smooth surfaces. The

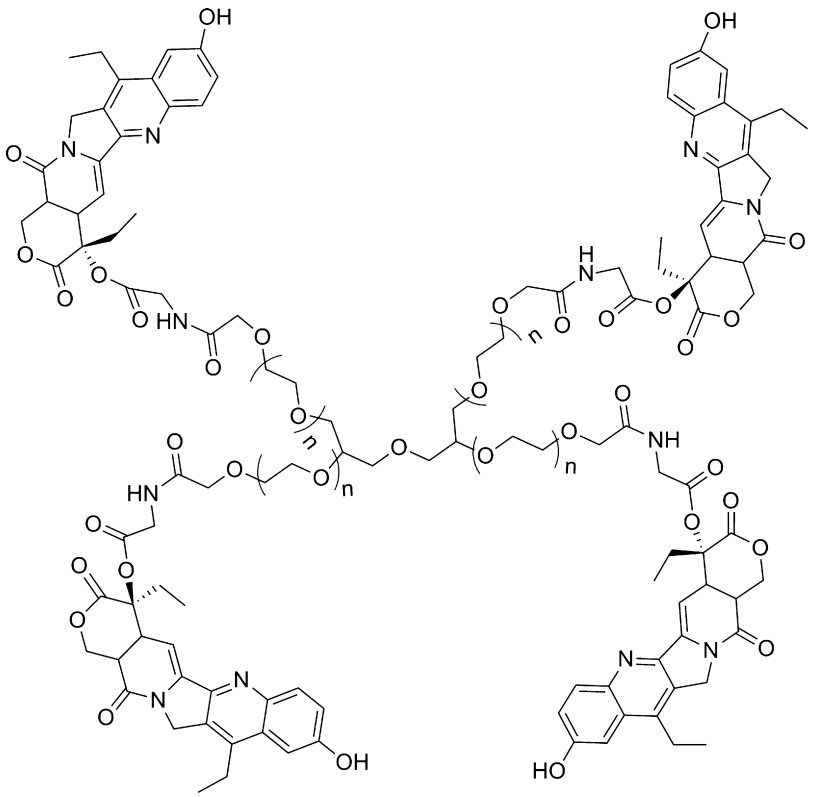


Fig. 17. Structure of ENZ-2208: 4arm-PEG-SN38.

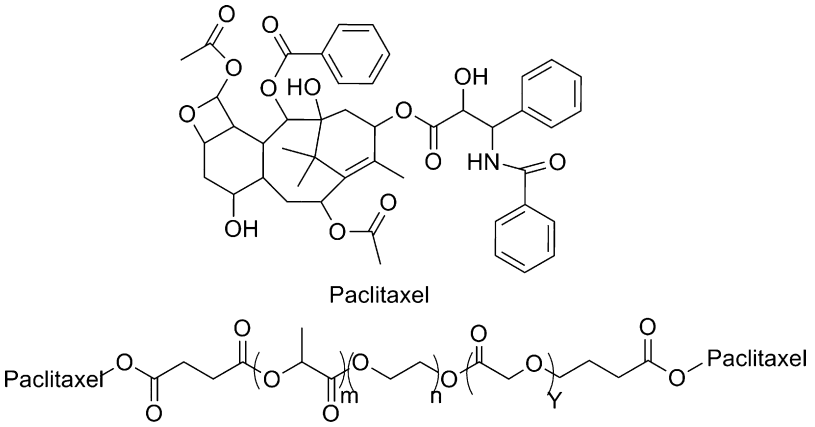


Fig. 18. Structure of the PLA-PEG-PLA/PTX conjugate.

amphiphilic property could be used in developing new drug delivery vehicles. The antitumor activity of the conjugate against woman Hela cancer cells was evaluated by the MTT method. The results showed that the PLA-PEG-PLA/PTX conjugates had high cytotoxicity against Hela cancer cells, motivating further developed as a clinical medicine. In the same year, Xie et al. [68] prepared another novel self-assembling paclitaxel conjugate [P(LGG-paclitaxel)-PEG-P(LGG-paclitaxel)], in which the parent polymer is P(LGG)-PEG-P(LGG). Drug release assay indicated that the release rate of paclitaxel from the conjugate increased with the reduction of pH. The conjugate exhibited visible cytotoxicity against rat brain glioma C6 cells, which implied that the paclitaxel was released from the conjugate without losing cytotoxicity, although its antitumor activity was lower than that of the native paclitaxel drug.

5.1.6. PEG-docetaxel

Docetaxel (DX), a derivative of paclitaxel, is one of the most effective antineoplastic drugs. However, its hydrophobicity and serious side effects limited clinical administration [69]. Xie et al. synthesized a DX conjugate with carboxyl-terminated copolymer monomethoxy-poly(ethylene glycol)-block-poly(L-lactide) (mPEG-PLLA) (Fig. 19) [69]. The conjugates were self-assembled into micelles in aqueous solution, which showed smooth surfaces with the size of less than 100 nm. The antitumor activity of the conjugate against Hela cells showed a value similar to that of the free drug DX.

PEGylated docetaxel (NKTR-105) was prepared using Nektar's innovative small molecule PEGylation technology (multi-arm PEG), previously applied in NKTR-102 (PEG-irinotecan) [70]. In a preclinical study, the half-life of docetaxel was approximately 11 h while that of NKTR-105 was up to 60 days (Fig. 20) [33,34]. NKTR-105 demonstrated significantly greater anti-tumor activity than drug docetaxel in mouse xenograft models of human non-small cell lung (H460) and colon (LS174T, LoVo) tumors. In this model, treatment with NKTR-105 produced a significantly greater ($p < 0.0001$) tumor growth delay (TGD) than docetaxel; 266% *vs.* 166% at maximum tolerated dose (MTD) (40 mg/kg NKTR-105, 30 mg/kg docetaxel) [71,72]. Greater TGD was also observed in LoVo tumor bearing mice at the MTD of 30 mg/kg: 128% for NKTR-105 *vs.* 64% for docetaxel [70]. This conjugate has entered phase I clinical studies enrolling approximately 30 patients who have failed all prior available therapies [70].

5.1.7. PEG-cisplatin

Cis-dichlorodiamminoplatinum (II) (cisplatin) is a widely used chemotherapeutic agent for treatment of various cancers, such as lymphoma, testicular cancer, and glioma [73–75]. However, the full therapeutic exploitation of cisplatin is limited by short blood circulation time and serious toxicity toward healthy tissues [75,76]. A targeted delivery of cisplatin to tumor cells would significantly reduce drug toxicity, thus improving its therapeutic index. Aronov et al. [77] synthesized several long-circulating PEGylated carboplatin analogs by conjugating the platinum moiety to folate-targeted PEG carriers, capable of utilizing the folate receptor-mediated endocytosis (FRME) (Fig. 21) [77]. Folate-targeted PEG conjugates enter the cells efficiently by the FRME pathway, but form relatively few DNA adducts, presenting higher IC_{50} values than carboplatin and their nontargeted analogs.

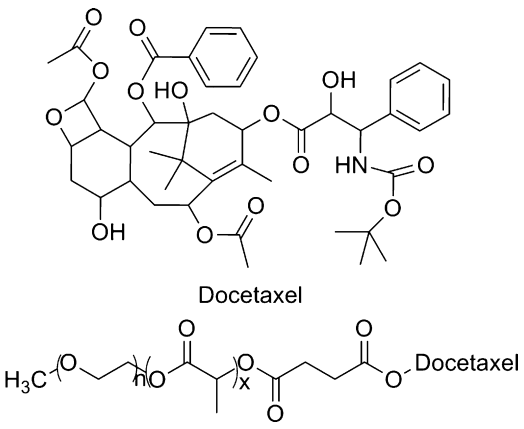


Fig. 19. Structure of MPEG-PLLA/DX.

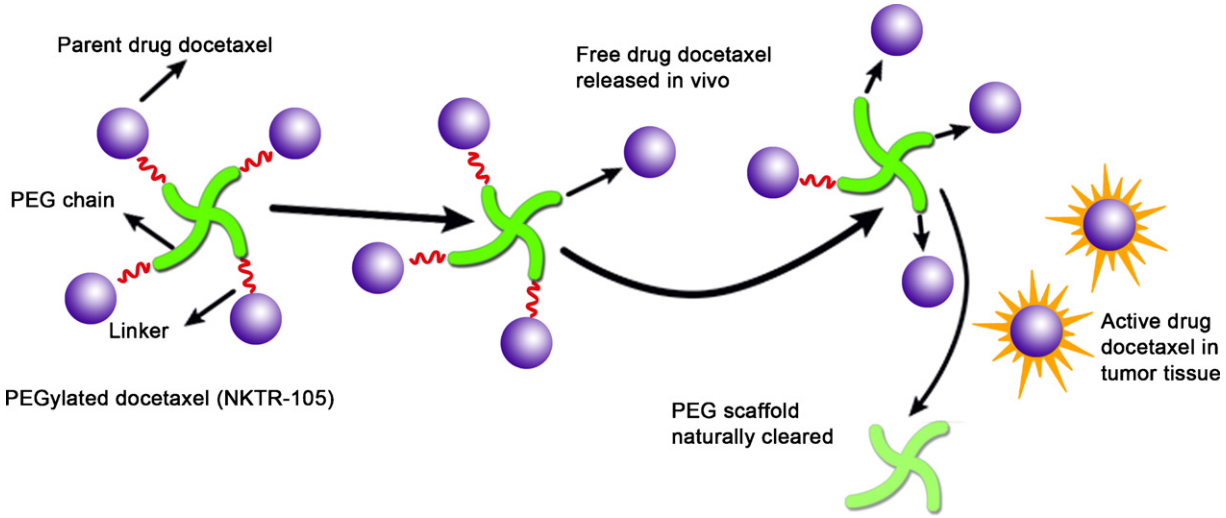


Fig. 20. The drug release process *in vivo* of multi-arm PEGylated docetaxel (NKTR-105) [72].

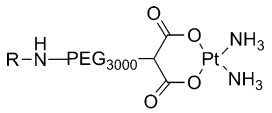


Fig. 21. Structure of PEG-dicarboxylato-platinum conjugates.

5.1.8. PEG-gemcitabine

Gemcitabine(dFdC), an antitumor agent structurally similar to AraC, is effective in the treatment of solid tumors and small-cell lung cancer. It has also been used in the treatment of pancreatic cancer [78]. However, gemcitabine possesses several defects, such as short plasma half-life, rapid metabolism and low selectivity toward tumor tissue, which hampers its widely clinical application. A series of different molecular weight PEGs modified gemcitabine prodrugs were synthesized by choosing folic acid as targeting agent (Fig. 22) [79]. As expected, these conjugates increased plasma half-life of gemcitabine by reducing its kidney clearance. The authors [79] also compared the antiproliferative activity of folic acid targeted with non-targeted conjugates and found that the targeted conjugates showed both higher antiproliferative activity and selectivity than the non-targeted ones in KB-3-1 cell lines.

5.1.9. PEG-wortmannin

Wortmannin is a non-competitive and irreversible inhibitor of phosphoinositide 3-kinase (PI3K), an important target for cancer chemotherapy [80,81]. Unfortunately, its toxicity, insolubility, and aqueous instability (through

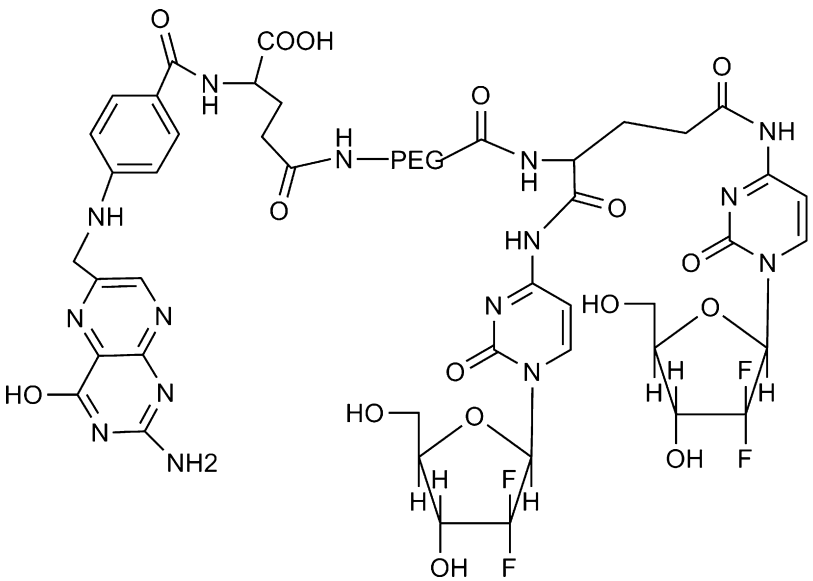


Fig. 22. Structure of folate-PEG-AD-$(dFdC)_2$.

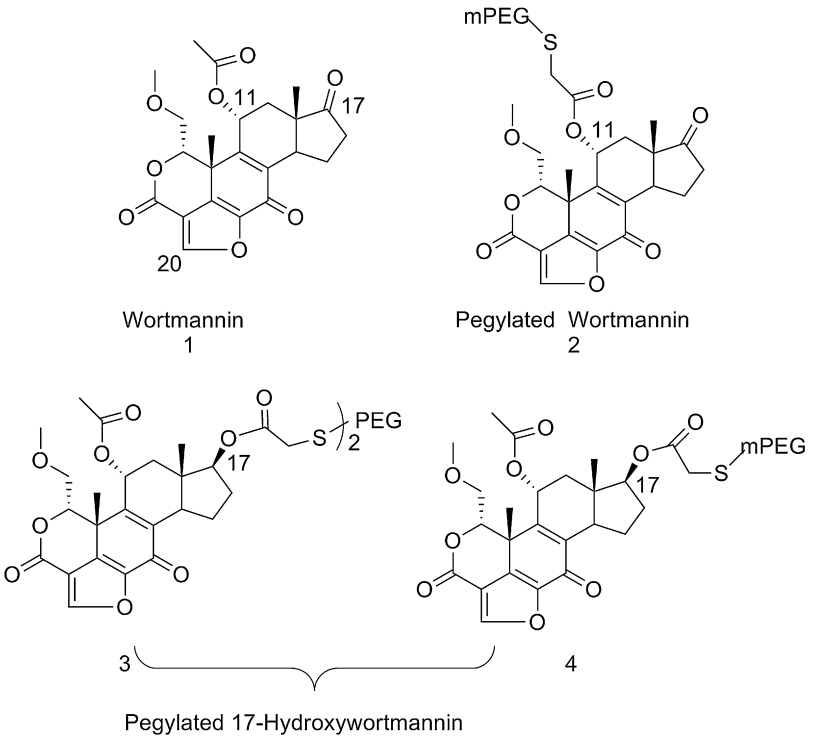


Fig. 23. Structures of PEGylated wortmannin and PEGylated 17-hydroxywortmannin analogs.

hydrolytic ring opening of its reactive furan ring) have limited its development into a viable anticancer agent. To improve therapeutic index, Zhu et al. applied PEGylation technology to wortmannin and 17-hydroxywortmannin [82]. Three PEGylated-wortmannin derivatives (Fig. 23) were synthesized under mild conditions and evaluated *in vitro* stability, as well as *in vivo* activity using PTEN-negative U87MG glioma cells in a xenograft model in athymic mice. The resulting conjugates overcome many deficits of the parent compounds, especially, conjugate 4, which greatly increased plasma stability and antitumor activity, as well as lower toxicity than the original drug, has been selected for further development [82].

5.1.10. PEG-pemetrexed

Pemetrexed (PM), a promising folated-based antimetabolite, has entered the clinical usage for the cancer treatment of mesothelioma and non-small-cell lung carcinoma [83]. However, it exhibits unfavorable physicochemical properties, such as poor water solubility and unstability in its original form of glutamic acid. An aqueous-soluble prodrug of PM was synthesized by conjugation with PEG (Fig. 24) [84]. The solubility of PEG_{4k}-Leu-PM and PEG_{20k}-Gly-PM was enhanced remarkably, to at least 650 and 125 mg/mL, respectively. PEG_{20k}-Gly-PM showed a considerable inhibitory effect on SMMC cell lines while the native drug exhibited a weak activity [84]. In the case of the HL-60 cell line, the inhibitory effect of prodrugs decreased with increasing PEG molecular weight. Accordingly, this study provided another feasible pharmaceutical form of PM in clinical application.

5.1.11. PEG-Lam-D

Lamellarin D (Lam-D) is a marine alkaloid with a wide range of biological activities, such as anti-proliferative activity against various tumor cell lines. Pla et al. synthesized a series of PEG conjugated Lam-D derivatives (2–9) to modulate the physicochemical properties and improving bioactivity of Lam-D (Fig. 25) [85,86]. PEG-Lam-D conjugates were tested in a panel of three human tumor cell lines (MDA-MB-231 breast, A-549 lung, and HT-29 colon) to evaluate their cytotoxicity. Compounds 2, 4, and 9 showed higher cytotoxicity than Lam-D in A-549 and MDA-MB-231 tumor cell lines, but displayed less cytotoxic activity in the BJ (skin fibroblast cell line) normal cell line, indicating that these compounds have better affinity to tumor cell lines [85].

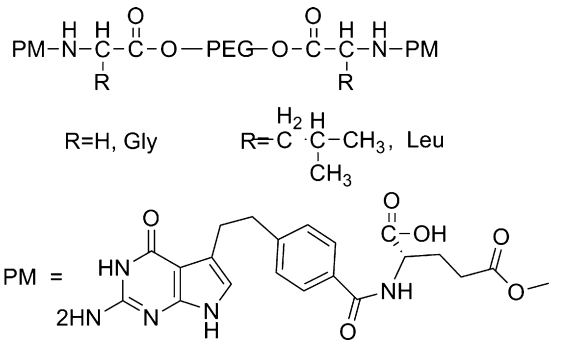


Fig. 24. Structures of PEGylated pemetrexed prodrugs.

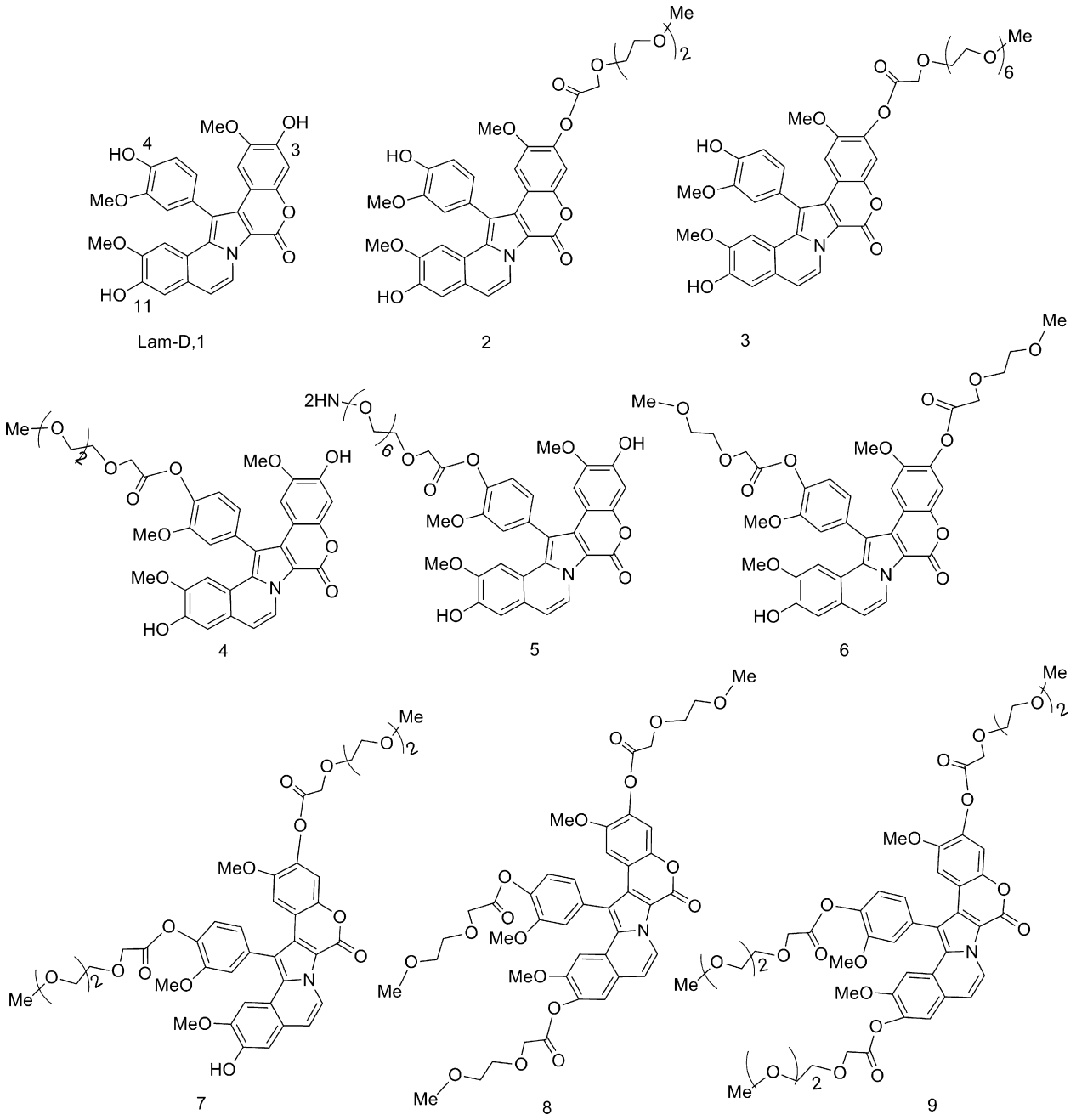


Fig. 25. Structures of Lam-D 1 and PEG derivatives 2–9.

Pla et al. [86] synthesized several Lamellarin D conjugates by covalent linking Lam-D to nuclear localization signal peptide and poly(ethylene glycol)-based dendrimer, respectively (see Fig. 26). PEGylated conjugates 1 and 2 showed much higher cytotoxicity in MDA-MB-231 cells than Lam-D alone. The cytotoxicity of Lam-D and its analogs (1, 2, and 4) was evaluated against BJ human skin fibroblasts, and a panel of three human tumor cell lines: A-549, HT-29, and MDA-MB-231. BJ skin fibroblasts were used in the study to evaluate the effects of the drug and its conjugates in normal cells. In this nontumoral cellular culture, cytotoxicity of conjugate 1 was similar to that of Lam-D, or even 2.4–4.9-fold less for 2 and 4. The cellular internalization quotient for the PEGylated derivatives 1 and 2 was higher than that of Lam-D in all cancer cell lines.

5.1.12. PEG-methotrexate

Methotrexate (MTX), a folic acid antagonist, is widely used clinically for the treatment of certain human cancers [87]. However, the efficacy of MTX in anticancer

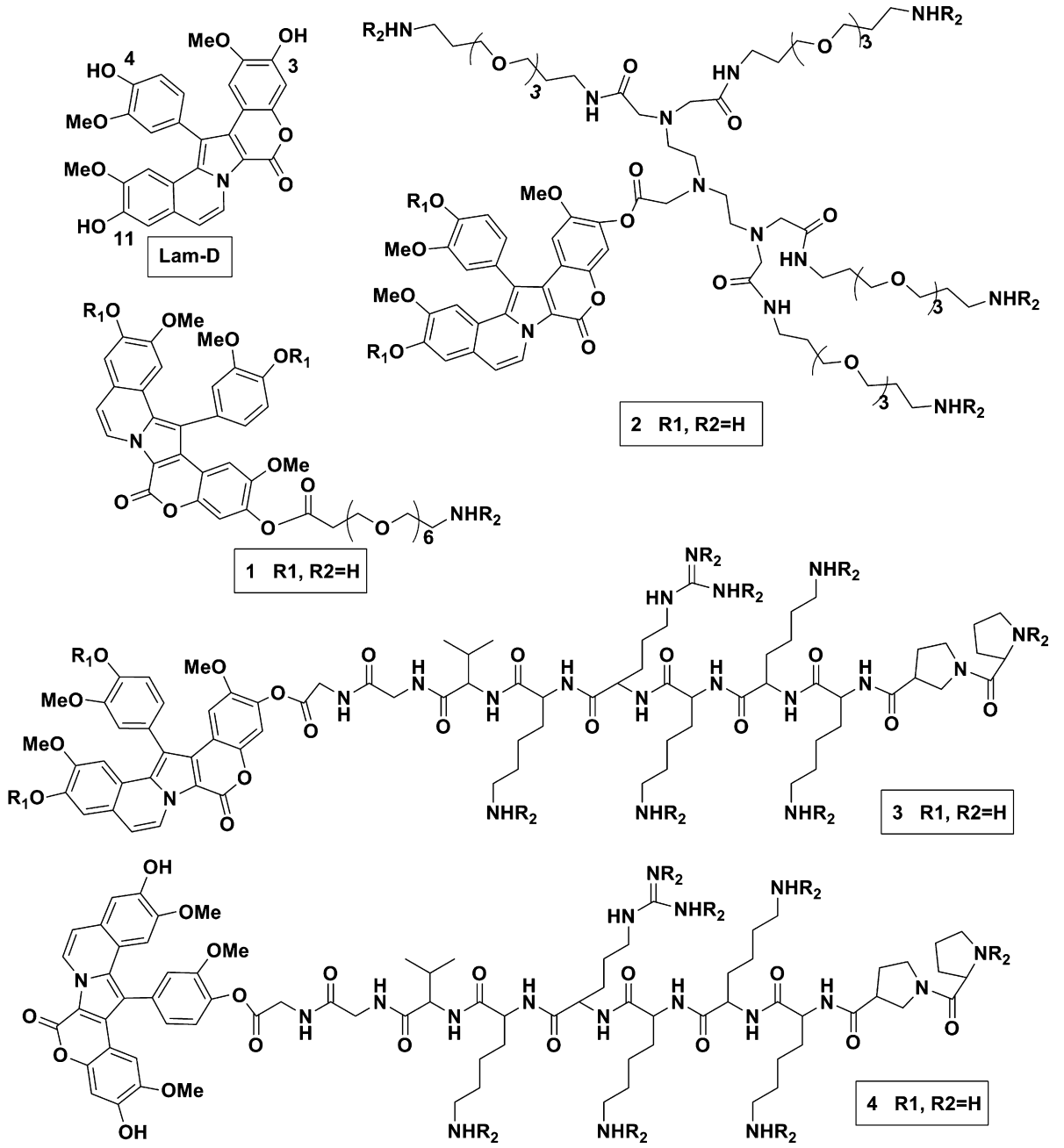


Fig. 26. Structure of Lam-D and its analogs.

chemotherapy is compromised by its very short plasma half-life, leading to drug resistance and causing nonspecific toxicities in normal proliferating cells. To overcome these shortcomings, Riebeseel et al. [88] synthesized a series of MTX-PEG conjugates with molecular weight ranging from MW 750 to 40,000 (see the reaction route in Fig. 27) [88]. The results indicated that release of MTX from polymer conjugates is not necessary for an effective interaction with the active site of dihydrofolate reductase. *In vitro* cytotoxicity evaluation revealed that the IC_{50} values of the tested compounds increased with the increased size of the drug–polymer conjugates. In contrast to the results *in vitro*, MTX-PEG conjugates with high molecular weight exhibited the highest antitumor activity *in vivo*. MTX-PEG_{40k} was superior to MTX at a dose of 20 mg/kg, demonstrating the same order of antitumor activity as MTX-HSA (20 mg/kg), a conjugate that is in phase II clinical trials as of the writing.

5.1.13. PEG-gambogic acid

Gambogic acid (GA), isolated from the gamboge resin of *Garcinia hanburyi* tree in Southeast Asia, has been used as a coloring material for painting [89]. Recent studies showed

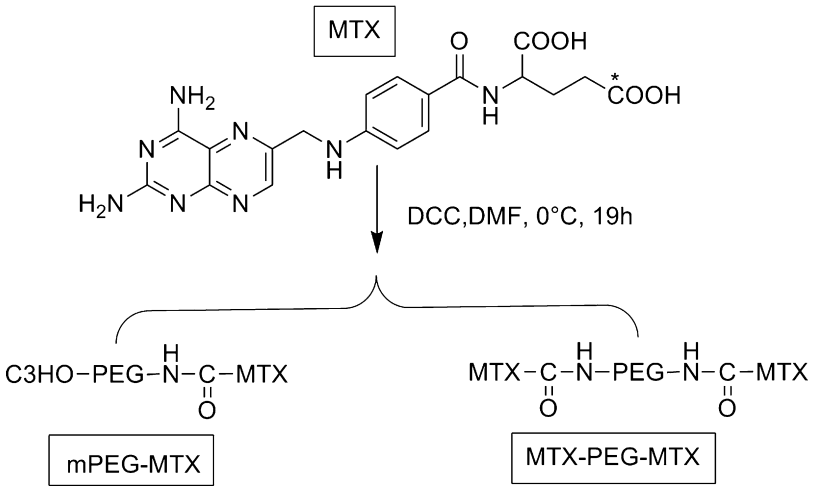


Fig. 27. Reaction pathway for the synthesis of MTX-PEG conjugates (atom with * is the active site).

that gambogic acid has potent cytotoxicity against various cancer cells both *in vitro* and *in vivo*. However, the low solubility in water and short half-time of GA restrict its clinical applications. To effectively improve its pharmacokinetic properties, enhance its therapeutic index and reduce adverse effects, a series of GA-PEG prodrugs with different molecular weight of PEG were synthesized by using a spacer of L-leucine (Fig. 28) [90]. The drug release from prodrugs was investigated under simulated *in vivo* conditions, predicting half-times ($t_{1/2}$) in plasma from 1.26 to 6.12 h. The PEG-GA conjugates showed higher stability with increasing molecular weight of PEG.

5.2. PEGylated antiviral drugs

5.2.1. PEG-saquinavir

Saquinavir (SQV), the first HIV-1-protease inhibitor (PI) approved by FDA, is an important drug commonly used for AIDS therapy [91]. However, therapeutic use of SQV suffers from several problems, such as low oral bioavailability of SQV in clinical formulations, cellular uptake/retention, metabolism/stability, and poor solubility. Gunaseelan et al. [92] prepared various poly(ethylene glycol) (PEG)-based prodrug conjugates of the SQV (conjugates 1–4, see in Fig. 29). *In vitro* stability studies showed that all conjugates were inactive until the ester bond was cleaved and active SQV was released. They evaluated anti-HIV-1 activity of the conjugates in MT-2 cells infected with HIV-1 Strain LAV/LAI (MOI of 0.01) and monitored the release of active drug from the conjugates using a novel HIV-1 protease inhibition assay (Table 3) [92]. The activity of conjugated SQV was reduced (ED_{50}, 900 nM) for conjugate 1, but restored for conjugate 2 (ED_{50}, 125 nM), conjugate 3 (ED_{50}, 15 nM) and conjugate 4 (ED_{50}, 62 nM). With addition of Cvs(R.I.CK-Tat9) ED_{50} of conjugate 3 reached the maximum achievable anti-HIV-1 activity (unconjugated SQV, ED_{50}, 15 nM). The cytotoxicity (LD_{50}) of all prodrugs was in the micromolar range [92]. The results also showed that all the PEGylated SQV conjugates were less toxic than free SQV, and the difference between the LD_{50} and ED_{50} suggested a favorable therapeutic index. While the *in vitro* assay presented a reliable indicator of SQV activity and release, the authors considered the major delivery advantages of conjugation can only be observed *in vivo*. Thus, absorption and elimination half-lives in plasma will have to be further studied in an *in vivo* model to evaluate delivery system performance.

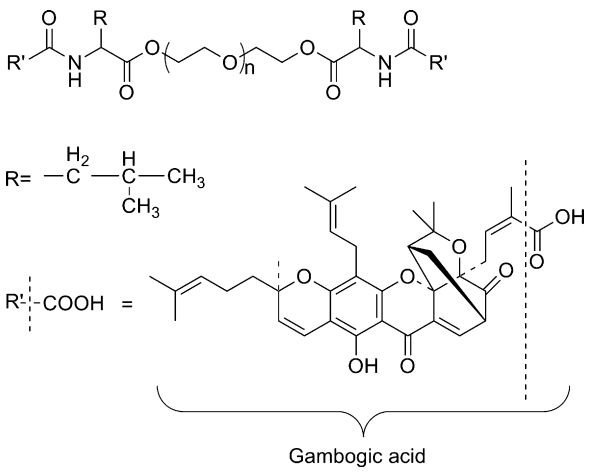


Fig. 28. Structures of PEG-Leu-GA prodrugs.

5.2.2. PEG-acyclovir

Acyclovir, a synthetic purine nucleoside analog derived from guanine, is used mainly for the treatment of herpes

Table 3
Anti-HIV-1 activity (ED_{50}) and cytotoxicity (LD_{50}) data for SQV and its prodrugs.

Compound	ED_{50} (μM)	LD_{50} (μM)	Therapeutic index LD_{50}/ED_{50}
SQV(MeSO3H)	0.015	25	1667
Conjugate 1	0.90	45	50
Conjugate 2	0.125	15	120
Conjugate 3	0.015	12.5	833
Conjugate 4	0.062	6.3	102

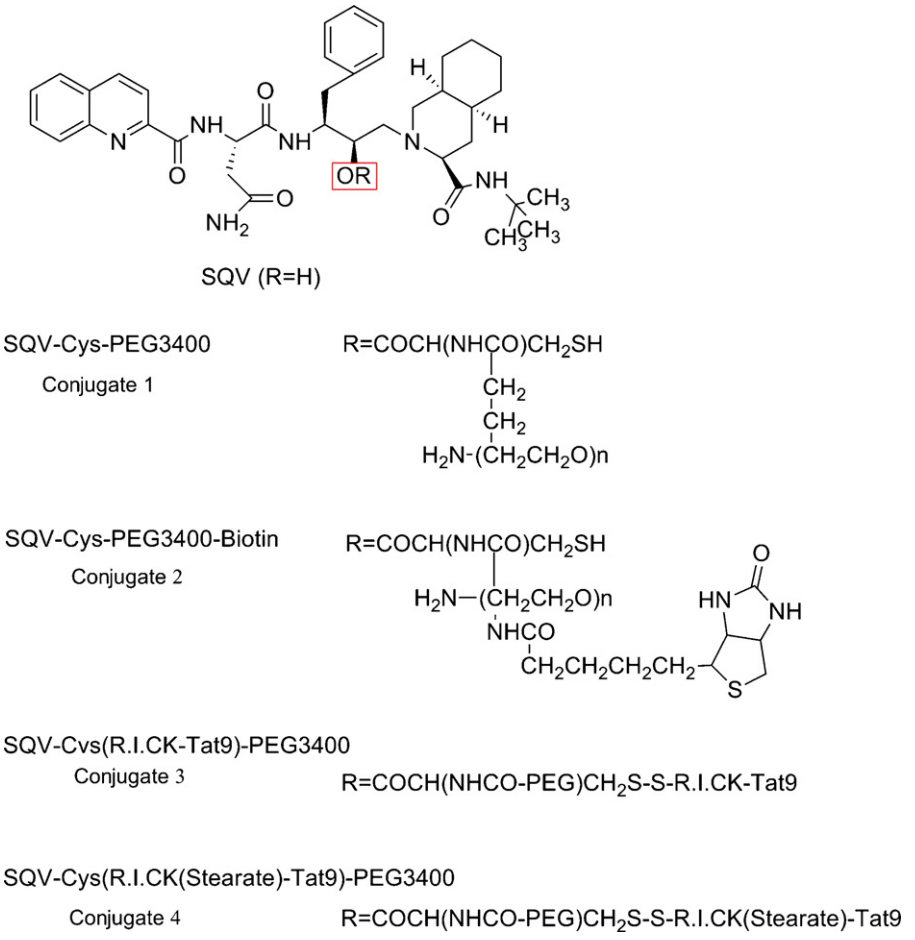


Fig. 29. The structures of PEGylated saquinavir.

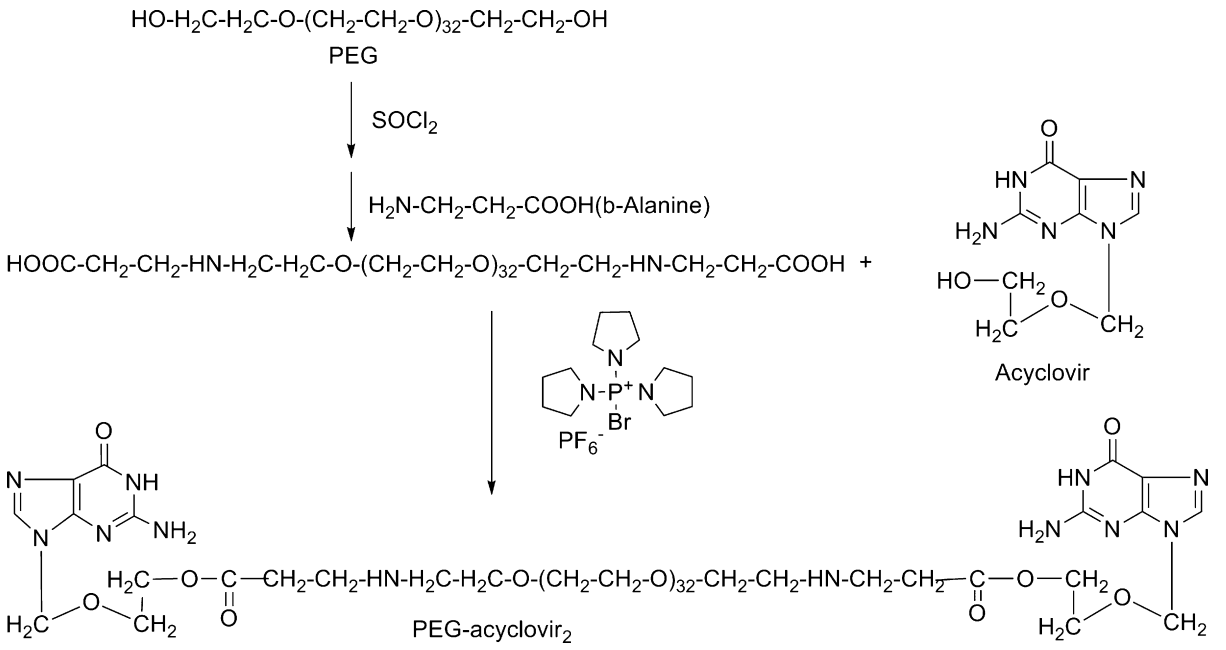


Fig. 30. Schematic synthetic procedure of PEG-acyclovir$_2$ conjugation.

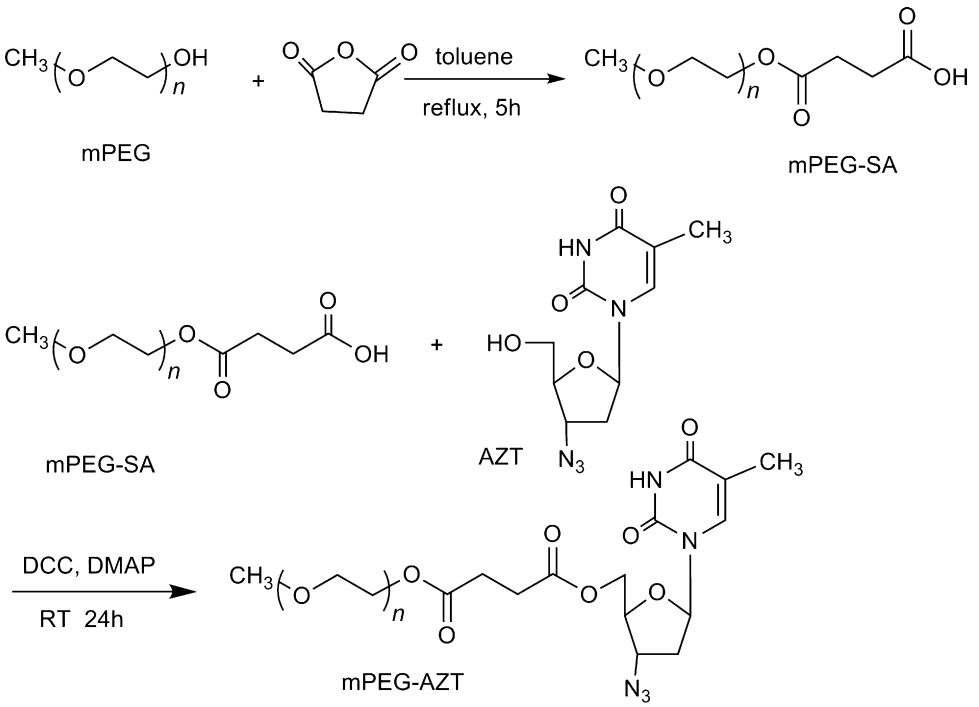


Fig. 31. Synthesis route of PEGylated conjugates of AZT.

zoster virus infections [93]. To avoid its poor solubility in water and increased chemical stability, Zacchigna et al. prepared two prodrugs of acyclovir using activated PEG (see Fig. 30) [94]. *In vitro* drug release studies demonstrated that the conjugates were stable in buffer solutions at pH 7.4 and 5.5. Pharmaceutical parameters *in vivo* of PEGylated acyclovir conjugates need to be further studied to evaluate improved performance in delivery systems.

5.2.3. PEG-zidovudine

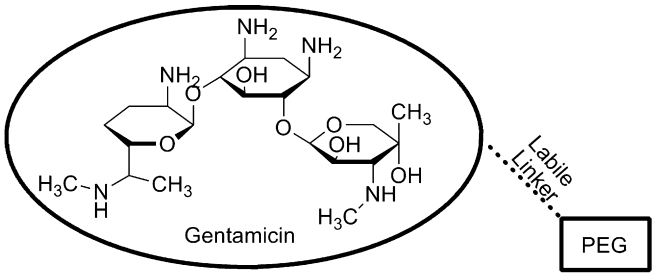
Zidovudine (AZT), the first anti-HIV drug inhibiting reverse transcriptase (RT), remains an important component in highly active antiretroviral therapy (HAART). However, the effectiveness of its therapy suffers from several clinical limitations, especially its short terminal half-life (1.2 h) and significant dose-related toxicity [25,95]. As mentioned in the preceding, our research group designed and synthesized a novel orally methoxy poly(ethylene glycol)-zidovudine (mPEG-AZT) conjugate (synthetic route: see Fig. 31). Pharmacokinetic properties were evaluated experimentally by oral administration in mice. Compared to free AZT, the absorption half-life ($t_{1/2ka}$) and elimination half life ($t_{1/2b}$) of AZT released from the conjugate were extended to 0.51 ± 0.03 h and 2.94 ± 0.24 h, respectively. The *in vitro* anti-HIV activities assay showed that the conjugate exhibited good inhibition of HIV-1, with an EC_{50} value of 0.0634 μM, but lower than that of free AZT. These results show that mPEG-AZT is capable of releasing the parent drug in a sustained form, potentially providing a feasible alternative to oral administration of AZT in a clinical setting [25].

5.3. PEGylated anti-inflammatory drug (PEG-gentamicin)

Gentamicin is a low-molecular-weight agent used to treatment many serious Gram-negative bacterial infections, but it reaches the urine within a short period after administration [96]. PEG-gentamicins were prepared by coupling gentamicin with variable length PEG-SH chain through labile linkers, *e.g.*, heterobifunctional agents (MAL-Fmoc-OSu and MAL-FMS-OSu) (Fig. 32) [97]. Following systemic administration to rats, PEGylated gentamicin conjugates released native active gentamicin with half-life 7–15-fold greater than that of non-derivatized gentamicin. Considering the effects of different molecular mass of the conjugates on clearance time of gentamicin *in vivo*, PEG_{40k}-linked gentamicin conjugates were the longer-lived species, but the residence time for PEG_{20k}-linked conjugates is similar. Therefore the authors [97] considered that the conjugate with molecular mass of 20 kDa for future application was economical and sufficient. Most important, the reversibly PEGylated gentamicin derivatives overcome the major drawback of irreversible PEGylation, namely, the loss of pharmacological potency.

5.4. PEGylated antimicrobial drug (PEG-amphotericin B)

Amphotericin B, an antifungal agent with a polyene structure, may be considered the antibiotic of choice for treatment of invasive and disseminated fungal infections [98]. However, its clinical use is limited by considerable treatment-associated toxicity, the most serious being nephrotoxicity. Sedlák et al. [99] prepared a conjugate of amphotericin B (AMB) using methoxypoly(ethylene glycol)



PEG { Mw=5000 / Mw=20000 / Mw=40000 }

Labile Linker { MAL-Fmoc-OSu : 9-hydroxymethyl-2-(amino-3-maleimidopropionate)fluorene-N-hydroxysuccinimide / MAL-FMS-OSu : 2-sulfo-9-hydroxymethyl-7-(amino-3-maleimidopropionate)-fluorene-N-hydroxysuccinimide }

Fig. 32. The structures of PEGylated gentamicin conjugates.

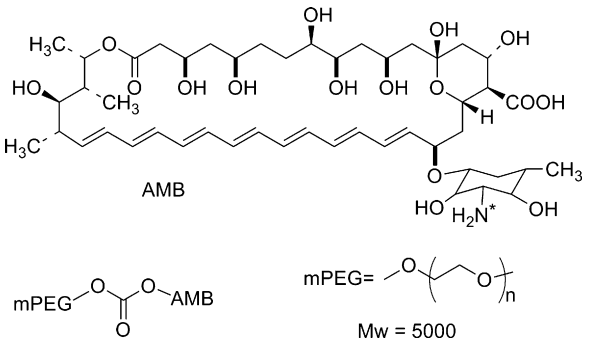


Fig. 33. The structures of PEG-amphotericin B conjugates.

(mPEG, Mw = 5000 g/mol) (Fig. 33). *In vitro* activity assays indicated that the activity of the mPEG-AMB was about 10 times higher than that of conventional AMB. In another study Sedlák et al. [100] synthesized new intravenous conjugates of amphotericin B (AMB) with poly(ethylene glycols) (PEG) (Mw = 5000, 10,000, 20,000) (see Fig. 34). All types of the conjugates are relatively stable in phosphate buffer at physiological conditions of pH 7.4 with half-lives ranging from 2 to 5 h. The LD_{50} values determined *in vivo* (mouse) are 20.7 mg/kg and 40.5 mg/kg for the conjugate 3b and 3a, respectively, which means that they are 6–11 times less toxic than free AMB (3.7 mg/kg).

5.5. PEGylated opioid antagonist

Naloxone, the opioid antagonist, is being developed to treat opioid-induced constipation (OIC) and other manifestations of opioid-induced bowel dysfunction (OBD) while preserving the central analgesic effect of opioids. In order to impede its crossing the blood–brain barrier, a PEGylated form of naloxol (PEG-naloxone) was obtained by Nektar Therapeutics (see Fig. 35) [101–104], in which, PEG

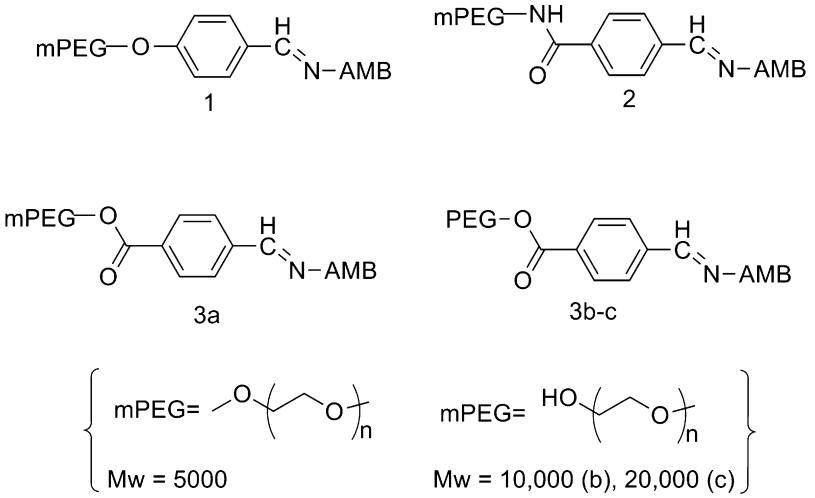


Fig. 34. The structures of PEGylated amphotericin B conjugates.

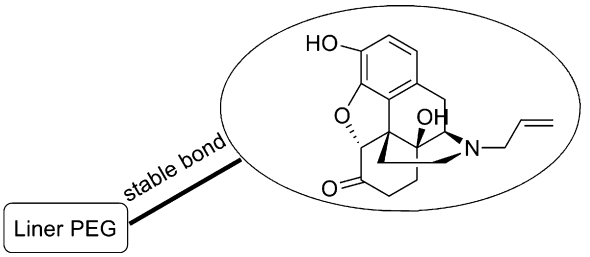


Fig. 35. Structure of PEGylated naloxone.

is conjugated to naloxol *via* a stable linkage to obtain novel orally administered NKTR-118. This conjugate is designed to selectively target peripheral opioid receptors and is under phase II clinical trials. In its phase I study, oral NKTR-118 was generally well-tolerated at doses up to 250 mg twice daily, with no serious or severe adverse events and at single doses up to 1000 mg. The phase II study for NKTR-118, in 208 pain patients experiencing OIC, was an international, multicentered, randomized, double-blind, dose-escalation, placebo-controlled trial. No treatment-related serious adverse events (SAE) for the 5 or 25 mg cohorts were observed. Only one patient experienced an SAE of hospitalization due to abdominal cramping in the 50 mg cohort [104–106]. NKTR-118 is currently in the phase III clinical program.

5.6. PEGylated immunosuppressant agent (PEG-tacrolimus)

Tacrolimus is a 23-member macrolide with potent immunosuppressive activity, isolated from *Streptomyces tsukubaensis* in 1984 [107]. To overcome the low solubility in water, polymer–tacrolimus conjugates were prepared by covalent linking tacrolimus to the water soluble polymer, methoxypoly(ethylene glycol) (mPEG) (see Fig. 36) [108]. These conjugates were converted again into tacrolimus by the action of enzymes in human liver homogenate. The half-lives of the conjugates are approximately 10 min in the homogenate, indicating that the esterified tacrolimus derivatives may be applicable as a prodrug for the immunosuppressant.

5.7. PEGylated other drugs

5.7.1. PEG-silybin

Silybin, the main component of silymarin, is an anti-hepatotoxic agent. But it presents numerous challenges to being launched as a drug due to its poor aqueous solubility

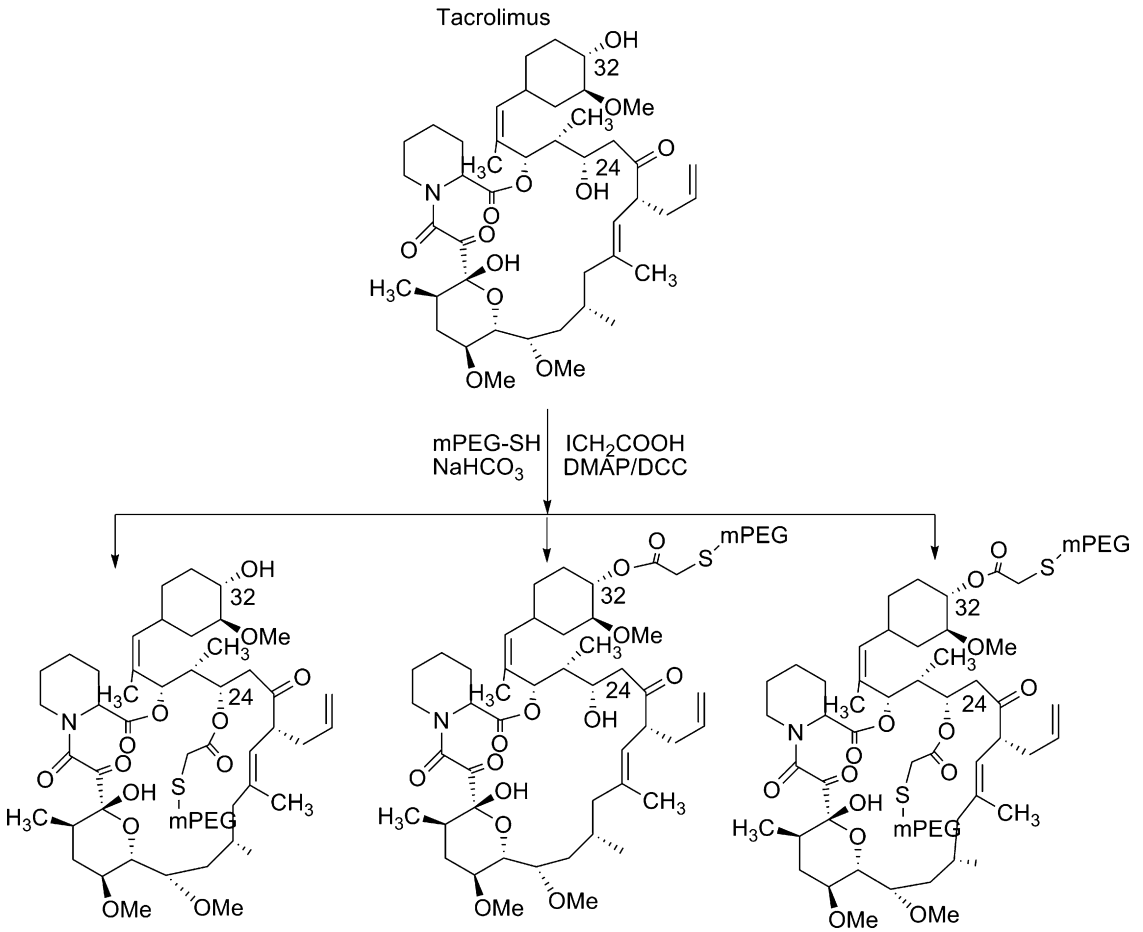


Fig. 36. Synthesis route of PEGylated conjugates of tacrolimus.

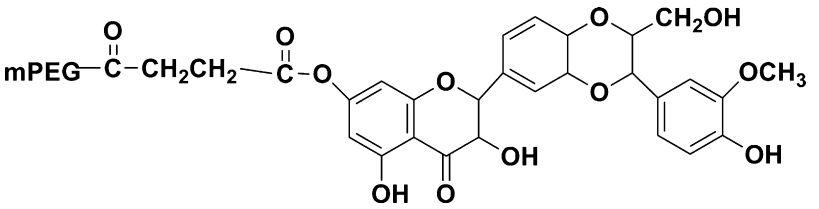


Fig. 37. Structure of PEGylated silybin.

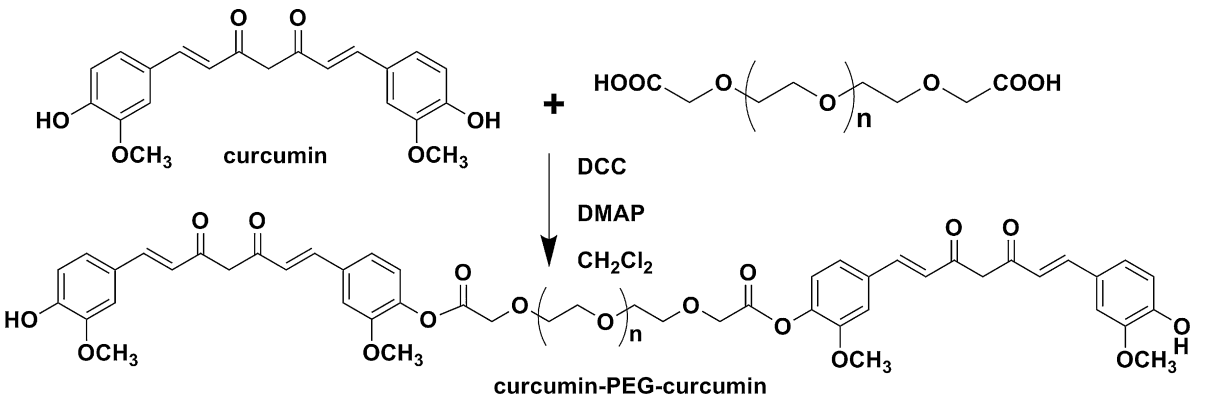


Fig. 38. Synthetic route of PEG-curcumin conjugation.

[109]. To solve the major problem in clinical application, a novel water-soluble silybin prodrug was synthesized by using a linear PEG and succinic ester linkage in 2008 (Fig. 37) [110]. The prodrug was evaluated for its drug loading capability and solubility, with results giving 6.65% and 800 mg/mL, respectively (the equivalent solubility of silybin was 52.5 mg/mL), indicating significantly increased water solubility of the prodrug in comparison with parent silybin, with a solubility of only 0.0401 mg/mL.

5.7.2. PEG-curcumin

Curcumin, a derivative from spice turmeric (*Curcuma longa*), has been widely studied for its antiinflammatory, antioxidant, and anticancer effects. However, curcumin is highly hydrophobic, and this feature prevents its clinical application [111]. To increase the solubility and targeted delivery, a water-soluble polyethylene glycol (PEG)-conjugated curcumin system was developed by Li et al. [111], in which curcumin is covalently linked to PEG (35 kDa) (synthetic route: see Fig. 38). PEGylated curcumin showed better solubility than parent drug curcumin and presented a profound inhibitory effect on cell growth at concentrations of 5 μM in pancreatic cancer cells, while curcumin is needed at 20 mM to exhibit a similar inhibitory effect [111]. The conjugate also showed synergistic effects with gemcitabine on cell growth inhibition and apoptosis in PANC-1 and AsPC-1 cells.

6. Conclusion and further perspective

The primary use of PEGylation has been to improve the physicochemical properties of large molecules, and its success promotes the application of this technology in small molecules, which may provide small molecule drugs with better physical characteristics and more favorable therapeutic effects. Small molecule drugs include a vast number of compounds that possess good potency, but with poor physicochemical properties and unfavorable pharmacokinetics, making them to be potential candidates for PEGylation. Linkers between PEG and small drugs are classified as stable linkage and releasable linkage, which create entirely new compounds or novel prodrugs, respectively. Generally, stable linkage conjugates need low molecular PEGs because large PEG may impede targeted binding of small active agents, while releasable linkage conjugates need large molecular PEGs to increase the circulating half-life of the parent drug. On the other hand, the architecture of PEGs also affects the efficacy of modified small drugs. Linear PEG is the most widely used for its simple synthetic steps and good water solubility. But the limitation of this system is its low drug loading capability. Branched PEG and forked PEG are used less frequently in small drug modification because of their lower flexibility and decreased solubility when the bound bind drug content is increased.

Although considerable research on the PEGylation of small agents has been reported in decades, no PEGylated drug has been approved to date. Some failed in preclinical evaluations due to reduced efficacy of therapy [67,68], and some were discontinued in early clinical trials owing to instability and toxicity [55,64,65]. Recently, multi-arm PEGs have received attention, not only because they facilitate high drug loading, but also because they exhibit good water solubility and express better therapeutic effects [112]. It offers a bright prospect for the PEGylation of small drugs. Three of the four PEGylated small drugs in clinical trial (NKTR-102, EZN-2208 and NKTR-105) utilize this dominant configuration, including NKTR-102 which is in phase III clinical trials.

Active targeting PEG system should also receive more attention in the future. By connecting with an active targeting moiety, PEGylated small drugs may present faster intra-tumor accumulation and higher intracellular concentrations of the parent drug. Active targeting successfully increases the differential selectivity of chemotherapeutic drugs and decreases the side effects, especially for antitumor drugs with cytotoxicity.

In conclusion, PEGylation provide a feasible strategy to enhance the therapeutic index of small molecule drugs. It can effectively increase the solubility of water insoluble compounds, lower the toxicity, generate a desired pharmacokinetics profile and enhance accumulation at the targeted site. Though PEGylation of small drugs meets many challenges in the development process, the recent successful evidence from drugs entered into the clinical trials brings a new perspective. Therefore, we are confident that PEGylated small drugs will become a realistic novel drug form applied in the clinical therapies.

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