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Paclitaxel nano-conjugated to polyhedral oligomeric silsesquioxane (POSS) nanoparticles as a novel water-soluble prodrug

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ABSTRACT

Condensed polyhedral oligomeric silsesquioxane (POSS) family of materials are very attractive as a nanoscale drug delivery system in cancer therapy. This work describes a novel method for conjugation of poorly soluble anticancer drug paclitaxel (PTX) to octa-aminopropyl polyhedral oligomeric silsesquioxane hydrochloride salt nanoparticles (OA-POSS NPs). The synthesized nanoconjugate of PTX and OA-POSS NPs (PTX_n-OA-$POSS_{8-n}$ prodrug) was fully soluble in aqueous solution as compared to the free PTX.

1. Introduction

Paclitaxel (PTX) is a member of the taxane family of compounds extracted from the bark of the Pacific yew tree *Taxus brevifolin* [1]. PTX is widely used as an anticancer drug against a wide range of cancers including breast, ovarian and lung cancers. However, the effectiveness of PTX as an anticancer drug is limited by its low solubility in aqueous physiological medium (5.56×10^{-3} g/L) as well as its low permeability in the tumor tissue [2]. In this regard, formulation based on a mixture of Cremophor EL and dehydrated ethanol (50:50, v/v) known as Taxol ® (Bristol-Myers Squibb) was developed. Due to its some adverse side effects corresponded to Cremophor EL and dehydrated ethanol, there is an immediate need for the expansion of new alternative PTX formulations [3]. Polymeric nanoparticles (NPs) and dendrimers have been used extensively for encapsulation or conjugation of anticancer agents to increase the drug's residence time in circulation and sustained drug release in the tumor tissue [4,5]. However, the use of polymeric NPs in cancer therapy is limited by polydispersity and dendrimers require a multistep synthetic process. NPs based on oligomers of symmetric nanostructured silsesquioxane (POSS), with their high water solubility and ease of functionalization, can overcome the shortcomings of polymeric NPs and dendrimers as a carrier in drug delivery [6]. Moreover, POSS NPs possess the ability to penetrate cell membranes which is important in drug delivery to tumor cells [7]. Here we report on a novel, one-step method to functionalize multiple arms of OA-POSS with PTX to produce the PTX_n-OA-$POSS_{8-n}$ nanoconjugate, as a water-soluble PTX prodrug.

2. Experimental section

2.1. Chemicals and instruments

The chemicals and instruments used in this work are described in Supporting Information file S1.

2.2. Synthesis of OA-POSS NPs

The procedure for the synthesis of OA-POSS NPs is provided in Supporting Information file S2.

2.3. Synthesis of PTX-COOH (Taxol-2′-hemisuccinate)

Taxol-2′-hemisuccinate was synthesized according to a procedure previously described [8]. Briefly, in a 25 ml round bottom flask containing 270 mg of Taxol (PTX) and 38 mg of succinic anhydride (SA) in 13 ml of CH_2Cl_2, 36 μL (10-fold molar excess) of dry pyridine was added at ambient temperature and stirred for 3 days. The progress of the reaction was monitored by TLC (ethyl acetate–hexane, 1:1, v/v). After the reaction, the solvent was evaporated under vacuum, the residue was dissolved in CH_2Cl_2 (5 ml), and the product (Taxol-2′-hemisuccinate) was purified by silica gel column chromatography (ethyl acetate–hexane, 1:1, v/v) to provide 258 mg of product (86%).

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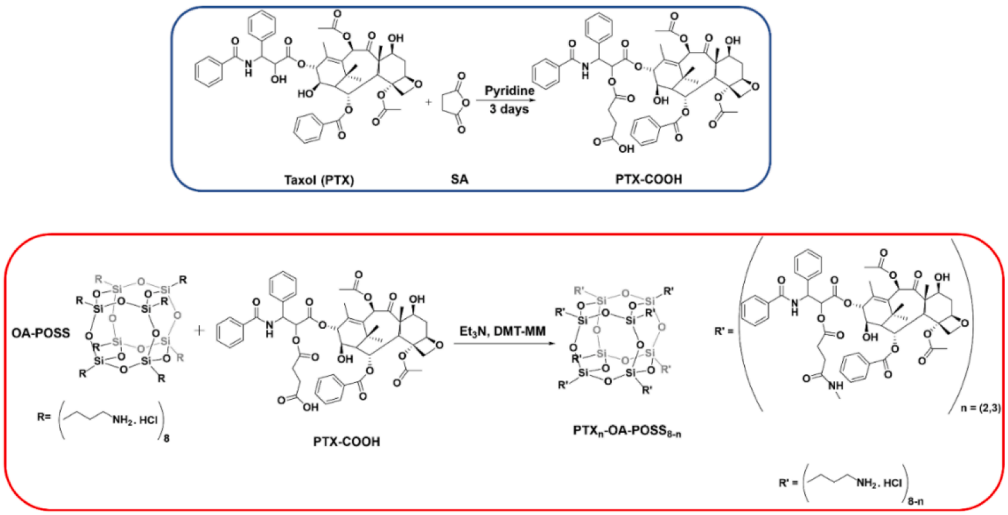

Fig. 1. The synthesis of water-soluble prodrug PTX_n-OA-$POSS_{8-n}$.

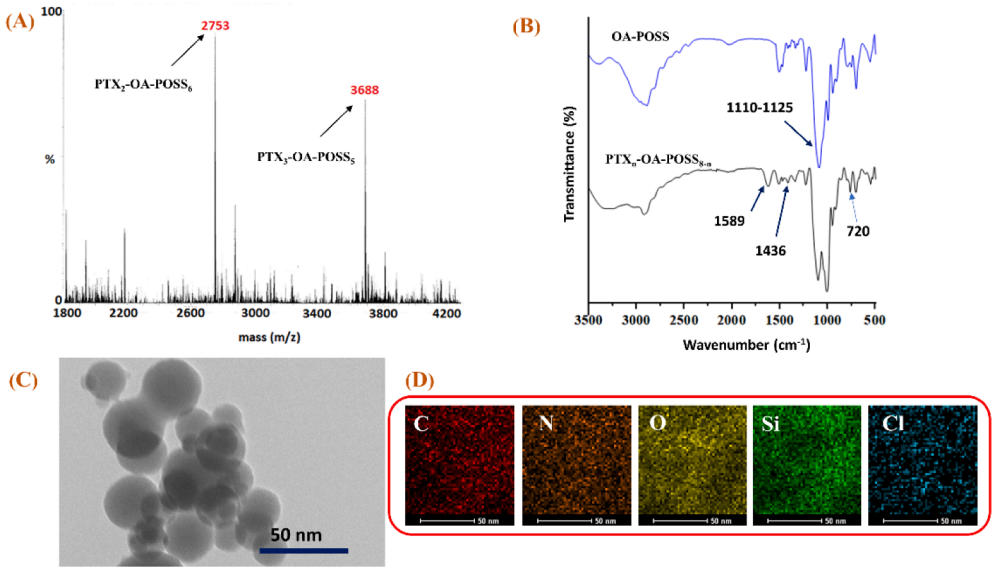

Fig. 2. (A) MALDI-TOF spectrum, (B) FTIR spectrum, (C) TEM image and (D) the corresponding TEM-elemental images of PTX_n-OA-$POSS_{8-n}$ nanoconjugates.

2.4. Synthesis of water-soluble PTX_n-OA-$POSS_{8-n}$ nanoconjugate

PTX-COOH (50 mg, 50 μmol), triethylamine (30 μL) and 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMT-MM, 20 mg, 60 μmol) were stirred in 2 ml dry DMSO until the solid was completely dissolved. To the mixture solution, OA-POSS (20 mg, 17 μmol) was added. The mixture was stirred at ambient temperature under argon atmosphere. After stirring for 24 h, the resulting mixture was poured into acetonitrile (45 ml) containing 0.1% HCl, vortexed, precipitated, and collected by vacuum filtration.

3. Results and discussion

3.1. Synthesis and characterization of materials

OA-POSS was synthesized by hydrolysis and condensation of 3-aminopropyltriethoxysilane (APTES) in acidic medium at 90 °C [9]. Paclitaxel (PTX) was reacted with succinic anhydride in the presence of catalytic amount of pyridine for functionalization with carboxy groups (PTX-COOH) and formation of an ester bond (cleavable by enzymes) at the C2́ position [8]. The PTX_n-OA-$POSS_{8-n}$ nanoconjugate was synthesized by conjugating the water-soluble OA-POSS NPs with PTX-COOH using DMT-MM as a coupling reagent, as depicted in Fig. 1.

The formation of OA-POSS, PTX-COOH and PTX_n-OA-$POSS_{8-n}$ was confirmed by MALDI-TOF MS. The presence of two sharp peaks at *m/z* 2753 and 3688 in the mass spectra (Fig. 2A), which were assigned to PTX_2-OA-$POSS_6$ (calculated theoretical molecular weight (MW) 2753) and PTX_3-OA-$POSS_5$ (calculated theoretical MW 3689) nanoconjugates, respectively, confirmed the successful conjugation of two or three PTX molecules to OA-POSS NPs. The molecular peaks at *m/z* 881 (calculated theoretical MW 881) and 954 (calculated theoretical MW 953.9) in MS spectra of Figs. S1 and S2 confirmed the formation of OA-POSS and PTX-COOH, respectively. The chemical composition of the synthesized PTX_n-OA-$POSS_{8-n}$ was studied by FTIR spectroscopy. The appearance of a new peak at 1589 cm^{-1} in the FTIR spectrum of PTX_n-OA-$POSS_{8-n}$ (Fig. 2B), which is related to the amide bond vibration, confirmed the conjugation of PTX-COOH to OA-POSS. The presence of two other new characteristic peaks at 1436 cm^{-1} and 720 cm^{-1}, which were assigned to the -C=C- stretching vibration of benzene ring of PTX [8], further confirmed successful conjugation of PTX-COOH to OA-POSS. In addition, the Si-O-Si

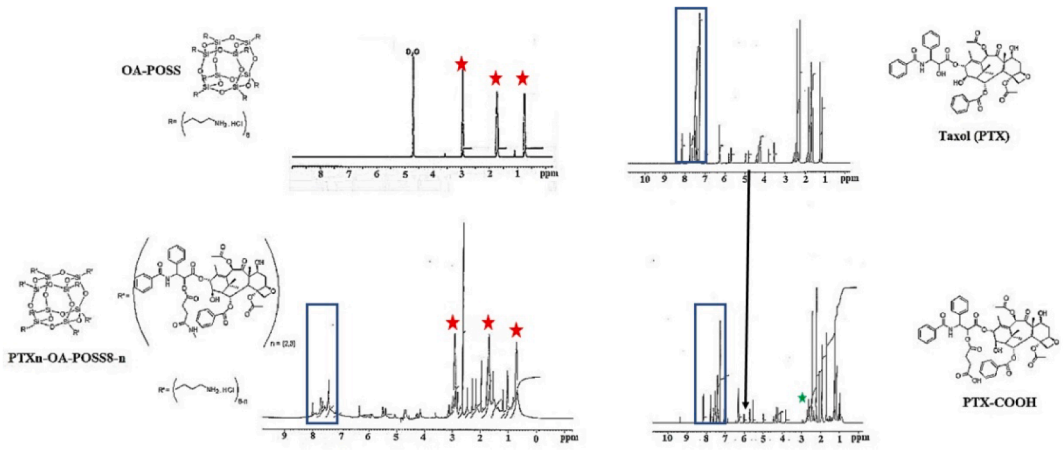

Fig. 3. ^{1}H NMR spectra of OA-POSS (in D_2O), PTX (in DMSO-d_6), PTX-COOH (in DMSO-d_6) and PTX_n-OA-$POSS_{8-n}$ nanoconjugate (in D_2O).

asymmetric stretching absorption between 1110 and 1125 cm^{-1} persisted as the most prominent peak of POSS cage in the spectrum of PTX_n-OA-$POSS_{8-n}$ [10]. The morphology of OA-POSS and PTX_n-OA-$POSS_{8-n}$ nanoconjugates was further studied by TEM analysis as shown in Fig. S3 and Fig. 2C, respectively. From Fig. S3, OA-POSS cores are apparent as dark spots with dimeters in the range of 5–10 nm [11]. However, the mean size of the PTX_n-OA-$POSS_{8-n}$ nanoconjugates increased due to high aggregation tendency. The nanoconjugates had a spherical shape with an average size of 20–30 nm. The elemental mapping images (Fig. 2D) reveal the existence of C, N, O, Si and Cl elements in PTX_n-OA-$POSS_{8-n}$.

^{1}H NMR spectra of OA-POSS, PTX, PTX-COOH and PTX_n-OA-$POSS_{8-n}$ are shown in Fig. 3. ^{1}H NMR spectrum of OA-POSS shows three sharp peaks with chemical shifts at 0.72, 1.67 and 2.90 ppm corresponding to Si-CH_2, –CH_2 and –CH_2-N- methylene groups, respectively [9]. The ^{1}H NMR spectrum of PTX-COOH showed the characteristic peaks centered at 2.6 ppm which corresponded to –CH_2CH_2- group of the succinic anhydride. In addition, the characteristic peak of 2́-H of PTX at 4.70 ppm was shifted to 5.95 ppm in PTX-COOH [8]. As compared with the ^{1}H NMR spectrum of OA-POSS, the aromatic chemical shifts at 7.2–8.0 ppm in the spectrum of PTX_n-OA-$POSS_{8-n}$ corresponded to the benzene ring of PTX. These results confirmed successful synthesis of the water-soluble PTX_n-OA-$POSS_{8-n}$ nanoconjugate.

For the stability study, PTX_n-OA-$POSS_{8-n}$ nanoconjugate was dissolved in distilled water and stored at room temperature. DLS result which is in good accordance with TEM results showed no remarkable change in the hydrodynamic particle size of PTX_n-OA-$POSS_{8-n}$ after 14 days (Fig. S4). This means that there is no physical association in PTX_n-OA-$POSS_{8-n}$ nanoconjugate, which guaranteed its high stability in distilled water. Finally, the solubility of PTX_n-OA-$POSS_{8-n}$ in water was examined and found to be 0.65 mg/mL which was significantly higher than PTX solubility in water (<0.004 mg/mL) [12].

4. Conclusion

A simple one-step reaction between OA-POSS NPs bearing amino groups and PTX-COOH in the presence of a coupling agent was successfully used to synthesize the PTX_n-OA-$POSS_{8-n}$ nanoconjugate as a novel water-soluble prodrug. MOLDI-TOF analysis of the product confirmed conjugation of two or three PTX molecules to OA-POSS NPs. The PTX_n-OA-$POSS_{8-n}$ nanoconjugates had a spherical shape and a size distribution in the range of 20–30 nm, as shown in TEM images. This work provides a POSS nano-building framework for modification of anticancer drugs to their water-solubility. Taken together, this work reports a novel and efficient method for the synthesis of water-soluble PTX_n-OA-$POSS_{8-n}$ nanoconjugate prodrug for application in cancer therapy. The evaluation of PTX_n-OA-$POSS_{8-n}$ as an anticancer prodrug against human cancer cells is in progress.

CRediT authorship contribution statement

Jafar Rezaie: Visualization, Investigation. **Nasrollah Jabbari:** Formal analysis, Investigation. **Sadegh Asghari Kalashani:** Visualization, Investigation. **Esmaiel Jabbari:** Formal analysis, Writing – review & editing. **Ali Akbari:** Conceptualization, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.matlet.2021.131013.

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