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Design, synthesis, and evaluation of water-soluble morpholino-decorated paclitaxel prodrugs with remarkably decreased toxicity

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ABSTRACT

Novel water-soluble paclitaxel prodrugs were designed and synthesized by introducing morpholino groups through different linkers. These derivatives showed 400–20,000-times greater water solubility than paclitaxel as well as comparable activity in MCF-7 and HeLa cell lines. The prodrug PM4 was tested in the S-180 tumor mouse model, with paclitaxel as the positive control. The results showed that PM4 had comparable antitumor activity as paclitaxel, with tumor inhibition of 54% versus 56%, and remarkably decreased toxicity. The survival rate of treated mice was 8/8 in the PM4 group, compared to 3/8 in the paclitaxel group.

Paclitaxel has been widely used in the clinic as an antitumor drug for lung, breast, and ovarian cancers.[1] It is a natural antimicrotubule diterpenoid isolated from the bark of the pacific yew tree (*Taxus brevifolia*).[2] However, due to its poor water solubility, paclitaxel is dissolved in dehydrated ethanol and Cremophor EL for clinical use, which causes serious side effects associated with hypersensitivity.[3,4] Numerous attempts to improve the water solubility of paclitaxel have been done by conjugating paclitaxel to some hydrophilic molecules, such as amino acids,[5,6] sugars,[7–9] malic acid,[10] polyethylene glycol,[11–13] dextran,[14] heparin,[15] and sulfonate.[16] Although most of these derivatives have much better water solubility than paclitaxel, some problems still remain, such as low stability, limited improvement in solubility, decreased activity, and high toxicity.

Morpholine is a hydrophilic molecule that could possibly improve the water solubility when introduced into paclitaxel, especially when the amino group is salified. Moreover, upon entering tumor tissues, the morpholino group would be protonated at the slightly acidic extracellular pH of tumors (6.5–7.2).[17–19] This would promote the interactions of prodrugs containing morpholino groups with negatively charged cell membranes and accelerate their endocytosis by tumor cells. Our previous work found that morpholino-decorated polymeric micelles exhibit higher cellular uptake at lower pH values (6.5–7.0).[20] Therefore, the toxicity of morpholino compounds to normal tissues, where the extracellular pH is 7.4, might be decreased.

Here, we report the design, synthesis, and evaluation of a series of new paclitaxel prodrugs by introducing morpholino groups through different linkers (Fig. 1). The primary aim of this work was to study the influence of morpholino groups on improving the water solubility of insoluble drugs like paclitaxel. Second, we wanted to examine whether the administration of morpholino derivatives of paclitaxel would prolong the survival time of tumor-bearing mice by decreasing the drug toxicity to normal tissues. In addition, the influence of different linkers on the stability and activity of the derivatives was studied. The linker of the ester bond in PM1 and the carbamate bond in PM2 may lead to different release rates of paclitaxel, possibly resulting in activity variation. In PM3, paclitaxel was conjugated to 4-(2-aminoethyl)morpholine (AEM) through a succinyl group; while in PM4, two AEM groups were introduced to reinforce the influence of the morpholino group. In PM4, a disulfide linker was incorporated because it has been reported that cleavage would occur only after cellular entry of the conjugate after encountering a high glutathione concentration (typically 15 mM intracellular compared to 15 μM extracellular).[21] The thiol resulting from glutathione cleavage has been reported to cyclize into the proximate carbonyl group of the linker, subsequently leading to the release of free paclitaxel.

Synthesis: The synthetic routes of the designed conjugates are given in Schemes 1–4. PM1 was obtained by directly reacting 2-morpholineacetic acid with paclitaxel through an ester bond in

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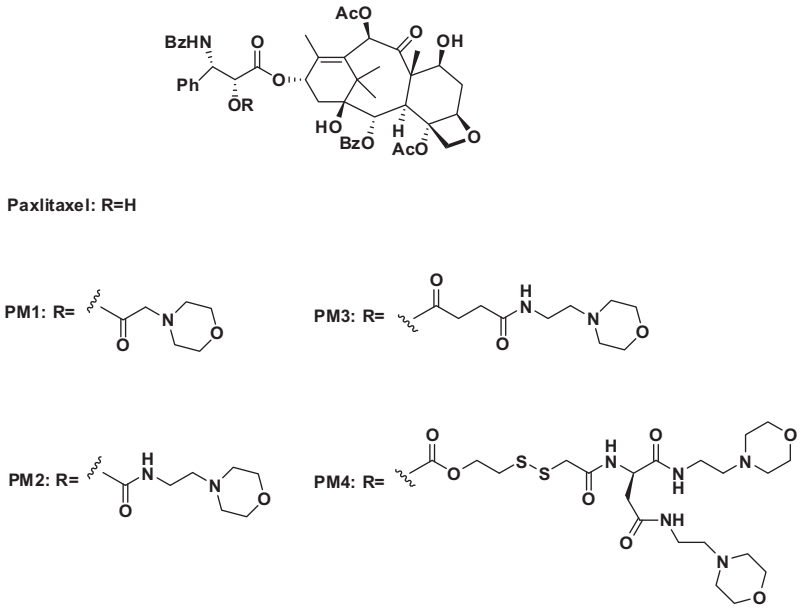
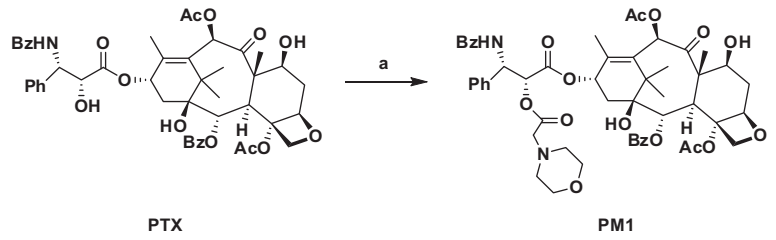
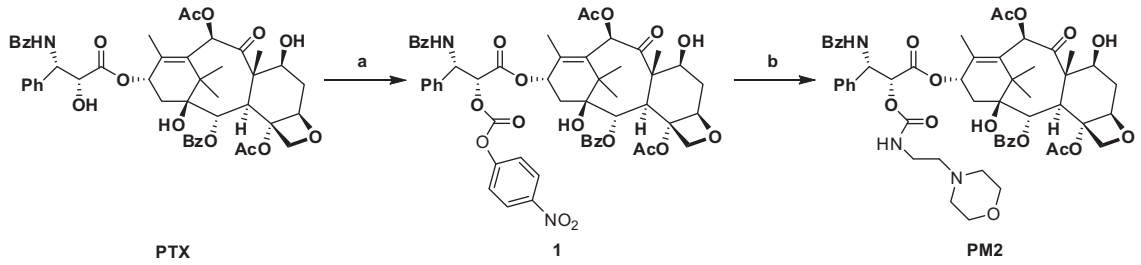


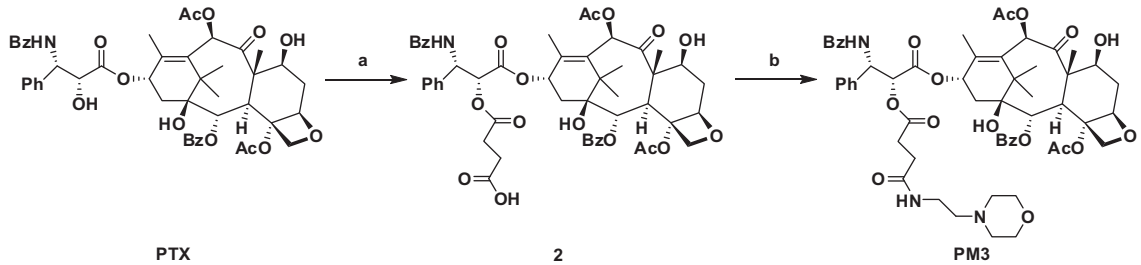
Figure 1. Structures of paclitaxel and its four prodrugs modified by morpholino groups.



Scheme 1. Synthesis of PM1. Reagents and conditions: (a) 2-morpholineacetic acid, EDCI, DMAP, DCM, rt.



Scheme 2. Synthesis of PM2. Reagents and conditions: (a) 4-nitrophenyl chloroformate, pyridine, DCM, rt; (b) 4-(2-aminoethyl)morpholine, DMAP, DCM, rt.



Scheme 3. Synthesis of PM3. Reagents and conditions: (a) succinic anhydride, pyridine, rt; (b) 4-(2-aminoethyl)morpholine, EDCI, HOBt, DMF, rt.

the presence of 1-(3-dimethylaminopropyl)-3-ethylcardodiimide hydrochloride (EDCI) and 4-dimethylaminopyridine (DMAP) in dichloromethane (DCM) at room temperature (rt). The reaction was completed in approximately 10 h. Next, DCM was evaporated,