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Synthesis and Evaluation of Water-Soluble Paclitaxel Prodrugs

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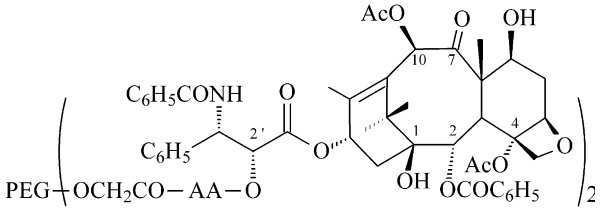
Abstract—A series of water-soluble 2′-paclitaxel prodrugs were synthesized by attaching paclitaxel to polyethylene glycol (PEG) through amino acid spacers. The prodrugs showed highly improved water solubility, enhanced in vitro cytotoxicity and in vivo antitumor activity compared with the native drug, paclitaxel.

Paclitaxel (Taxol®), a diterpenoid derived from the bark of the Pacific yew tree *Taxus brevifolia*,[1] is an anticancer agent with a unique mechanism of action. Its clinical activity has been well established for several neoplastic diseases.[2,3] However, owing to its poor aqueous solubility (0.25 $\mu g \cdot mL^{-1}$),[4] paclitaxel is often administrated in a vehicle containing both Cremophor EL (polyethoxylated castor oil) and ethanol, which has been demonstrated to cause a number of side effects.[5–7] Numerous attempts have been made to improve the aqueous solubility and pharmacological functions of paclitaxel.[8,9] The prodrug strategy has been considered as a promising way[10] in terms of improving the aqueous solubility while keeping the inherent pharmacological functions unaltered. To date, several reports of paclitaxel prodrugs have appeared. Deutch[11] and Zhao[12] reported the synthesis of 2′-paclitaxel esters with amino acid moieties but the derivatives were unstable compounds that readily converted to paclitaxel. Nicolaou[13] and co-workers synthesized a series of paclitaxel prodrugs and demonstrated that strong electron withdrawing substituent in the α-position of the paclitaxel ester may accelerate the hydrolytic cleavage of paclitaxel. Greenwald[14] proposed PEG (especially high molecular weight PEG) as solublizing agent to render paclitaxel greater aqueous solubility and demonstrated the importance of maintaining $t_{1/2}$(circulation) $> t_{1/2}$ (hydrolysis). However, serious drawbacks still exist in these paclitaxel prodrugs such as the poor stability, limited improvement in solubility or too stable feature leading to the difficulty in releasing the parent drug under the normal physiological conditions. These disadvantages severely limit their clinical usage.

The aim of the current study was to synthesize a new kind of water-soluble paclitaxel prodrugs, in which PEG was used as solublizing moiety to circumvent the delivery barrier of paclitaxel and amino acids as spacers to modulate the release of paclitaxel from prodrugs. Besides, we try to examine whether conjugation of paclitaxel to PEG through different amino acid spacers would influence the activity as compared with native paclitaxel. Both PEG and amino acids are biocompatible and display no undesired side effects, which offers the paclitaxel prodrugs the great potential of further clinical applications. The schematic structures of the new paclitaxel produgs are as follows.



The preparation of 2′-taxol prodrugs starting from PEG diol was outlined in Scheme 1.

Typically, PEG diol **1** (azeotropyed with toluene prior to use) was reacted with succinic anhydride in the presence of catalytic amount of pyridine to introduce the functional carboxy groups.[15] The reaction proceeded

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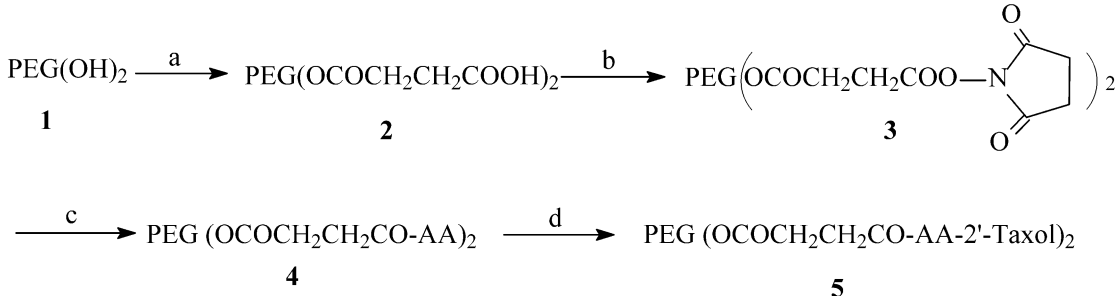


Scheme 1. (a) Succinic anhydride, $CHCl_3$, pyridine, 60 °C; (b) NHS, DCC, DMF, 0 °C; (c) amino acid, DMF, rt; (d) paclitaxel, DCC, DMAP, CH_2Cl_2, 0 °C.

smoothly in $CHCl_3$ and gave the PEG-diacid **2** in 95% yield. The carboxy groups in **2** make it possible to attach the amino acids to the backbone of PEG. However, the yield of the direct reaction of amino acids with compound **2** was quite low (30–40%). Two methods called 'active amide approach' and 'active ester approach' were therefore proposed to accelerate the reaction and increase the yields. The latter was found more efficient in our study. The diacid **2** was then treated with *N*-hydroxyl succinic anhydride (NHS) in DMF at 0 °C using DCC as coupling agent and converted to the active ester **3** in 90% yield. The product **3** was reacted with different amino acid to give PEG–amino acid conjugate **4** in a yield of about 80%. Finally, paclitaxel was joined to product **4** by a standard coupling procedure with the aids of DCC and DMAP to give **5** in 50% yield. The pure targeted product **5**, 2′-paclitaxel ester, could be obtained by recrystallization in Allyl Alcohol. The novel prodrugs **5a**–**f** synthesized in this way were listed in Table 1.

The in vitro biological activities of the paclitaxel and prodrugs were evaluated in three tumor cell lines, including a human breast cancer (MCF-7), a human non-small lung cancer (PG-49) and a murine leukemia (L1210) cell lines (Table 2). All drugs were tested at different incubation time, refer as 72 and 96 h in Table 2.

As anticipated, the cytotoxicities of prodrugs and native paclitaxel were all time-dependent. The IC_{50} values at 96 h were 5–10 times lower than those at 72 h, confirming that sufficient exposure time is essential for the drugs to kill tumor cells effectively. Among the six prodrugs, **5b**, **5c** and **5f** possessed significant cytotoxicities in the two human cancer cell lines. These prodrugs exhibited comparable (72 h exposure) or superior activity (96 h exposure) compared to the parent paclitaxel. In the case of murine L1210 cell line, **5c** and **5f** showed comparable activity to paclitaxel in 96 h. On the other hand, prodrugs **5d** and **5e** were essentially less active than paclitaxel. The behavior of compound **5a** in this test is different from others. In all three cell lines, it was always less active than paclitaxel in 72 h exposure and as active as paclitaxel in 96 h exposure. This may be due to the slow release rate of paclitaxel from the kind of conjugate possessing glycinate moiety.[16] These preliminary results revealed that the paclitaxel in the prodrugs has being released efficiently from the prodrugs into the medium and the amino acid spacers could affect the activity of prodrugs. Among these prodrugs, **5b**, **5c** and **5f** showed superior activity to paclitaxel against MCF-7. We selected compound **5f** as a candidate for further in vivo investigation.

Prodrug **5f** dissolved in saline and intravenously administrated against human breast cancer xenograph Bcap-37 (grown sc in athymic mice) and B16 melanoma (implanted subcutaneously in mice). Paclitaxel dissolved in cremophor/ethanol (52.7%/47.2%) was used as a positive control and solvent cremophor/ethanol (52.7%/47.2%) used as common control. A summary of the preliminary results is shown in Table 3.

Table 1. Prodrugs of paclitaxel

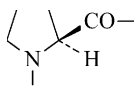
Compd	–AA–
5a	$–NHCH_2CO–$
5b	$–NHCH_2CH_2CO–$
5c	$–NH(CH_2)_5CO–$
5d	$–NHCH(CH_2CH(CH_3)_2)CO–$
5e	$–NHCH(CH_2CH_2SCH_3)CO–$
5f	

Table 2. Cytotoxicity[a] of paclitaxel and produgs in three tumor cell lines (IC_{50}, nM)[b]

Compd	MCF-7		PG-49		L1210	
	72 h	96 h	72 h	96 h	72 h	96 h
Paclitaxel	2.87	0.50	2.14	0.31	0.58	0.09
5a	3.51	0.58	3.15	0.30	0.87	0.10
5b	0.46	0.06	1.79	0.22	2.32	0.53
5c	1.53	0.22	3.10	0.17	1.65	0.05
5d	5.31	1.08	4.31	0.78	3.08	0.72
5e	9.53	7.91	13.5	5.73	1.42	0.64
5f	1.53	0.21	2.00	0.17	2.22	0.05

[a]Cytotoxicity was determined by the MTT assay.
[b]IC_{50} (nM), concentration required to inhibit cell growth by 50% on continuous exposure time of 72 or 96 h.

Table 3. Activity of prodrugs and paclitaxel against Bcap-37 xenograft and B16 melanoma[a]

Tumor	Group	Dose[b] (mg/kg)	Body weight changes on day 20 (%)	No. of toxic deaths	Tumor inhibition (%)[c]
Bcap-37	Control	—	+48.8	0/12	—
	Paclitaxel	8	+16.9	0/6	78.02
	Prodrug 5f	20	+29.5	0/6	71.43
		10	+37.6	0/6	56.81
		5	+39.3	0/6	48.35
B16 melanoma	Control	—	+34.2	0/20	—
	Paclitaxel	10	+7.8	2/10	71.40
	Prodrug 5f	20	+22.8	0/10	56.26
		15	+25.8	0/10	48.13
		10	+26.2	0/10	39.00
		5	+30.2	0/10	29.05

[a]Prodrugs and paclitaxel were given daily (iv×7). Treatment initiated at 24 h after implantation.
[b]Equivalent dose of paclitaxel.
[c]Tumor inhibition = (1−average tumor weight of treated group/average tumor weight of control group)×100.

As shown in Table 3, the prodrug **5f** (dose 20 mg/kg) exhibited similar tumor inhibitory activity compared with native paclitaxel (dose 8 mg/kg) in the case of human breast cancer xenograft. Besides, **5f** exhibited excellent antitumor activity against the Bcap-37 human tumor xenograft than the B16 melonomen. The tumor inhibition values for Bcap-37 xenograft reached 71.43% whereas that for B16 melonomen was only 56.26% at dose level 20 mg/kg. Similar manners existed in other cases. Judging from the body weight changes, the prodrug seemed to be less toxic than formulated paclitaxel. For example, body weight increases of mice in treated group were two times higher than those in positive control group at the dose of 20 mg/kg of paclitaxel equivalent. Moreover, two of ten mice died due to the toxicity of formulated paclitaxel in the treatment of B16 melanoma whereas no toxic death was found in the prodrugs treatment groups. The less toxicity of the prodrug might be attributed to the lower release rate of paclitaxel from the prodrugs compared to that from the cremophor micelles, resulting in the lower peak plasma level. In addition, the toxicity associated with the cremophor and ethanol vehicle might also be reduced due to the improved water solubility of the prodrug.

Here we reported an efficient synthetic route for the preparation of water-soluble paclitaxel prodrugs with amino acids spacers and revealed that the spacer may potently affect the activities of prodrugs. The optimal prodrug **5f** demonstrated remarkably reduced toxicity, enhanced in vitro cytotoxicity and comparative in vivo antitumor activity compared with native paclitaxel. These preliminary results indicated that the kind of prodrugs hold excellent properties and deserve attention. Further studies about the prodrugs including the in vivo anticancer activity at their optimal dose (OD), the maximum tolerated dose (MTD) in mice, the release mechanism of paclitaxel from prodrugs and the pharmcokinetics features will be submitted later.

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References and Notes

1. Wani, M. C.; Talyor, H. L.; Wall, M. E.; Coggon, P.; McPhail, A. T. *J. Am. Chem. Soc.* **1971**, *93*, 2325.
2. Einzig, A. I.; Wienik, P. H.; Schwartz E. L. In *New Drugs, Concepts and Results in Cancer Chemotherapy*; Muggia, F. M., Ed.; Kluwer Academic: Boston, 1992.
3. Rowinsk, E. K.; Cazenave, L. A.; Donehower, R. C. *J. Natl. Cancer Inst.* **1990**, *82*, 1247.
4. Vyas, D.; Wong, H.; Crosswell, A. R.; Casazza, A. M.; Knipe, J. O.; Mamber, S. W.; Doyle, T. W. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 1357.
5. Fjallskog, M. L.; Frii, L.; Bergh, J. *Lancet* **1993**, *342*, 876.
6. Rowinsk, E. K.; Eisenhauer, E. A.; Chaudry, V.; Arbuck, S. G.; Donehower, R. C. *Semin. Oncol.* **1993**, *20*, 1.
7. Dorr, R. T. *Ann. Pharmacother.* **1994**, *28*, S11.
8. Sharma, U. S.; Balasubramanian, S. V.; Straubinger, R. M. *J. Pharm. Sci.* **1995**, *84*, 1223.
9. Sharma, A.; Straubinger, R. M. *Pharm. Res.* **1994**, *11*, 889.
10. Mellado, W.; Magri, N. F.; Kingston, D. G. I.; Garcia-Arenas, R.; Orr, G. A.; Horwitz, S. B. *Biochem. Biophys. Res. Commun.* **1984**, *124*, 329.
11. Deutsh, H. M.; Glinski, J. A.; Hernandez, M.; Haugwitz, R. D.; Narayanan, V. L.; Suffness, M.; Zalkow, L. H. *J. Med. Chem.* **1989**, *32*, 788.
12. Zhao, Z.; Kingston, D. G. I.; Crosswell, A. R. *J. Natl. Prod.* **1991**, *54*, 1607.
13. Nicolaou, K. C.; Riemer, C.; Kerr, M. P.; Rideout, D.; Wrasidlo, W. *Nature* **1993**, *364*, 464.
14. Greenwald, R. B.; Gilbert, C. W.; Pendri, A.; Conover, C. D.; Xia, J.; Martinez, A. *J. Med. Chem.* **1996**, *39*, 424.
15. Zalipsky, S.; Gilon, C.; Zilkha, A. *Eur. Poly. J.* **1983**, *19*, 1177.
16. Pendri, A.; Conver, C. D.; Greenwald, R. B. *Anti-Cancer Drug Des.* **1998**, *13*, 387.