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HIGHLY WATER SOLUBLE TAXOL DERIVATIVES: 2'-POLYETHYLENEGLYCOL ESTERS AS POTENTIAL PRODRUGS

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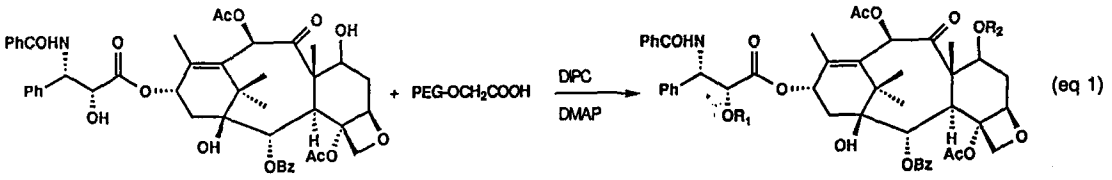
Abstract. *2' and 7-polyethylene glycol esters of taxol were prepared and found to be essentially water soluble. The rates of hydrolysis of these compounds were measured under neutral, acidic and physiological (basic) conditions. The half-lives of O and N substituted 2'-esters are short enough to permit their use as prodrugs.*

Water soluble prodrugs of taxol (**1**) have been the object of several recent serious investigations.[1-5] Thus far, these approaches have all been based on ester hydrolysis of poorly soluble ($\leq$10 mg/ml) prodrugs. The purpose of those studies was to circumvent the use of potentially antigenic solubilizing agents such as Cremophor EL which is currently being employed for taxol infusion.[6] The most recent work in this direction is the study by Nicholaou and coworkers [4] who provide precisely designed taxol esters which possess strong electron withdrawing substituents (such as alkoxy) in the α-position of the ester in order to accelerate hydrolytic cleavage. Additional modification also provided anchiomeric rate enhancements. The solubility of these prodrugs varied from $<$0.1 to 1.2 mg/mL and were reported to have half-lives $>$8.3 h at pH 7.5 and 37°C. A shorter *in vitro* half-life (1.5-2 h) was demonstrated for one compound when human plasma was employed.[7]

Polyethylene Glycol (PEG)[8], an amphiphilic macromolecule, [9] imparts greater aqueous solubility to conjugates of hydrophobic organic compounds when the molecular weight of PEG is 2 kD or greater.[10] By incorporating PEG as the α-alkoxy group of an acid, taxol esters with highly enhanced water solubility should be produced. Thus, PEG 5000 carboxylic acid [11] (**2**) was coupled to taxol [12] in 90-95% yield using diisopropyl carbodiimide (DIPC) and dimethylaminopyridine in methylene chloride to give exclusively the 2'PEG ester of taxol (**3a**).



(eq 1)

1 2

3a; R_1= $PEGOCH_2C(O)$-, R_2=H
3b; R_1= $CH_3OCH_2C(O)$-, R_2 = H
3c; R_1 = $CH_3OCH_2C(O)$-, R_2 = $PEGOCH_2C(O)$-
3d; R_1 = H, R_2 = $PEGOCH_2C(O)$-
3e; R_1= $PEGNHC(O)CH_2CH_2C(O)$-, R_2=H
3f; R_1=$PEGOC(O)NHCH_2C(O)$-, R_2=H

The solubility of **3a** was estimated to be $\geq$ 666 mg/mL at ambient temperature, and as shown in Figure 1, stability was observed for prolonged periods in Phosphate-saline buffer (PBS) at pH 5.8. Dissolution of **3a** in PBS at pH 7.0 and 7.4 (physiological pH) resulted in slow release of taxol ($t_{1/2}$=5.5 $\pm$ 0.5h, pH 7.4) as was expected.[13]

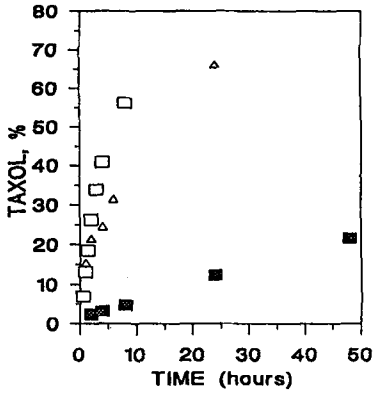


Figure 1. Kinetics of taxol release from 3a at 37°C in PBS buffer of pH (□) 7.4, (△) 7.0, and (■) 5.8.

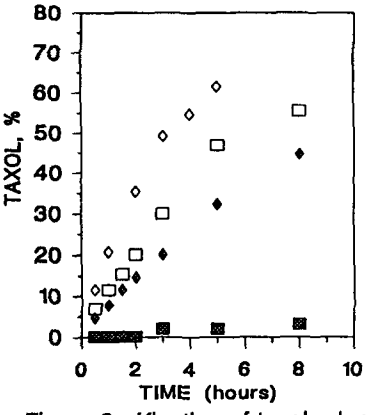
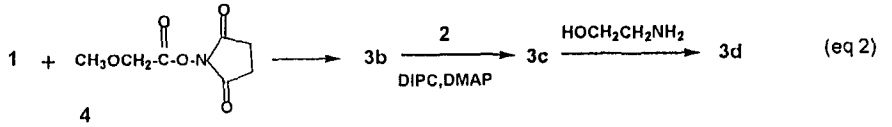


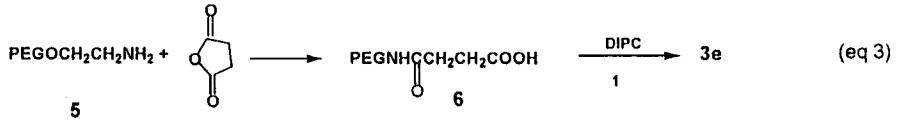
Figure 2. Kinetics of taxol release from various esters at 37°C in PBS buffer of pH 7.4 for (□) 3a, (■) 3d, (◊) 3e, and (♦) 3f.

Preparation of the 7-PEG 5000 ester **3c** was accomplished employing the 2'-methoxyacetate (MAc) ester (**4**)[10] as a blocking group (equation 2).



(eq 2)

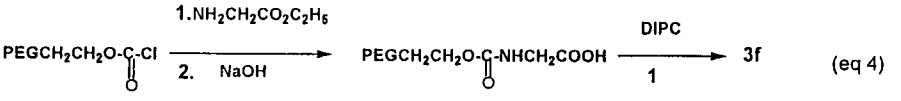
Treatment of **3c** with excess ethanolamine produced the desired ester **3d** with no indication of ammonolysis occurring at the 7-position. Ester **3d** was also found to be virtually water soluble. Kinetic studies of the hydrolysis of **3d** were performed in similar fashion to those of the 2'-ester **3a** (see Figure 2), and clearly demonstrate the stability of esters in the hindered 7-position.[14] Therefore, the greater reactivity reported for taxol 7-γ-carboxy-α-alkoxy-esters [4] must be due exclusively to neighboring group participation. Additional evidence for this effect was provided by the γ-carboxamido-2'-taxol ester **3e** prepared from acid **6**[15] in similar fashion to **3d**.



5 6 (eq 3)

The half-life of **3e** was determined to be 3.0 ± 0.5 h at pH 7.4 and constitutes the shortest half-life found for any of the esters examined in this study. (Figure 2).

For purposes of extending the half-life of taxol esters, the use of less election withdrawing substituents in place of oxygen was explored. Reaction of PEG 5000 chloroformate[16] (**7**) with glycine ethyl ester followed by saponification resulted in a 90% yield of the PEG substituted acid **8**.



7 8 (eq 4)

Esterification of taxol (**1**) with **8** using DIPC resulted in a 92% yield of **3f**. The kinetics of hydrolysis of **3f** are presented in Figure 2. As anticipated, a longer half-life was observed ($t_{½}$ = 8.5 ± 1.0 h) at pH 7.4.

Both the 2'-esters (**3a**, **3e**, and **3f**) and the 7'-ester (**3d**) were tested for *in vitro* activity according to published protocols [17, 18] using the murine leukemia cell lines P388 and L1210 (Table 1). The 2'- ester prodrugs had IC_{50} values essentially the same as unmodified taxol (5-18 nM) for both taxol-sensitive strains

indicating that taxol had been released from the prodrug. The 7'-ester exhibited reduced cytotoxic activity, but still retained an IC_{50} in the 270 nM range.

TABLE 1

Activity of Taxol and Pro-Taxols against taxol-sensitive (/O) P388 and L1210 leukemias *in vitro*.

	INHIBITORY CONCENTRATION [IC_{50},(nM)]	
Chemical Series	P388/O	L1210/O
Taxol	6	6
3a	15	17
3d	270	270
3e	11	9
3f	5	18

P388/O, and L1210/O cell lines were obtained from Southern Research Institute (Birmingham, AL) and were grown in RPMI 1640 supplemented with 10% FBS. For cytotoxicity assays, PEG prodrugs were dissolved in water while DMSO was employed for taxol. Cells were added to serial dilutions of test samples and were incubated at 37°C in a humidified incubator with 5% CO_2 for 3 days. Cell growth was measured by the addition of alamarBlue (Alamar Biosciences, Inc., Sacramento, CA) and the plates were incubated a further 4 hours at 37°C. The absorbances were determined using a microtiterplatereader at 570 nm with automatic subtraction of the background at 630 nm.

The kinetics of taxol release in rat plasma was also determined. Prodrug **3e** was dissolved in water, added to the plasma (EDTA treated), and incubated at 37°C for various times. The plasma was extracted with ethyl acetate[19], and the concentration of taxol was determined by reverse-phase HPLC using a phenyl column. The $t_{1/2}$ in plasma was 1.1 hours (Figure 3). The shorter half-life for the PEG-ester bond compared to pH 7.4 buffer is expected, since rodent plasma contains high non-specific esterase activities.

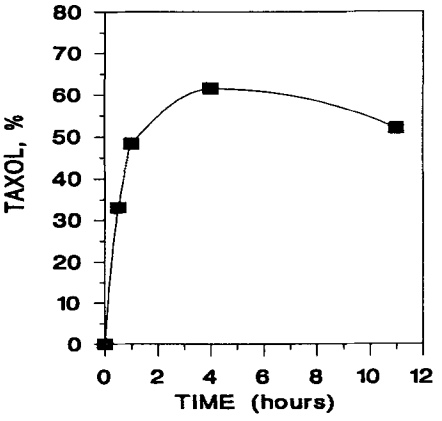


Figure 3. Kinetics of taxol release from 3e in rat plasma at 37°C.

Thus, the use of PEG for not only solubilizing taxol, but to afford controlled release of the drug over various periods of time, has been accomplished and may provide the first viable method of aqueous delivery of this important drug.

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REFERENCES

1. Deutsch, H.M.; Glinski, J.A.; Hernandez, M.; Haugwitz, R.D.; Narayanan, V.L.; Suffness, M.; Zalkow, L.H. *J. Med. Chem.*, **1989**, 32, 788-792.
2. Zhao, Z.; Kingston, D.G.I. *J. Nat. Products*, **1991**, 54, 1607-1611.
3. Mathew, A.E.; Mejillano, M.R.; Nath, J.P.; Himes, R.H.; Stella, V.J.; *J. Med. Chem.*, **1992**, 35, 145-151.
4. Nicolaou, K.C.; Riemer, C.; Kerr, M.P.; Rideout, D.; Wrasidlo, W., *Nature*, **1993**, 364, 464-466.

5. Vyas, D.M.; Wong, H.; Crosswell, A.R.; Casazza, A.M.; Knipe, J.O.; Mamber, S.W.; Doyle, T.W. *Bioorg. Med. Chem. Lett.*; **1993**, 3, 1357-60.

6. Rowinsky, E.K.; Onetto, N.; Canetta, R.M.; Arbuck, S.G. *Sem. Oncology*, **1992**, 19, 646-662.

7. Human plasma is known to contain unspecified amounts of esterases.

8. All polyethylene glycol derivatives discussed in this paper are terminated on one end of the polymer with a methyl group. The IUPAC nomenclature for the general polymer is α-methyl-ω-hydroxypolyoxy-(1,2-ethanediyl). We find it more convenient to refer to this material as PEG.

9. Harris, J.M., *Poly(Ethylene Glycol) Chemistry*, Ed. by Harris, J.M., Plenum Press, New York, **1992**, Chapter 1.

10. Greenwald, R.B.; Pendri, A.; Bolikal, D.; - manuscript submitted for publication.

11. a. Veronese, F.M.; Caliceti, P.; Pastorino, A.; Schiavon, O.; Sartore, L.; Bianci, L.; Scolaro, L.M., *J. of Controlled Release*, **1989**, 10, 145-154.

b. Gehrhardt, H.; Mutter, M.; *Polymer Bulletin*, **1987**, 18, 487-493.

12. Taxol was supplied by PHYTO Pharmaceuticals, San Carlos, CA.

13. The difference in $t_{1/2}$ for PEG ester **3a** compared to those reported for more insoluble alkoxyesters may be due to the use of DMSO as co-solvent [4].

14. Mellado, W.; Magri, N.F.; Kingston, D.G.I.; Garcia-Arenas, R.; Orr, G.A.; Horwitz, S.B., *Biochem. Biophys Res. Commun.*, **1984**, 124, 329-336.

15. Shadle, P.J.; Koths, K.E.; Moreland, M.; and Katre, N. *U.S Patent*, **1989**, 4,847,328.

16. S. Zalipsky, *U.S. Patent*, **1992**, 5,122,614.

17. Kelland L.R.; Abel, G., *Cancer Chemother. Pharmacol.* **1992**, 30, 444-450.

18 Waud, W.R.; Gilbert, K.S.; Harrison, S.D.; Griswold, D.P. *Cancer Chemother. Pharmacol*, **1992**, 31, 255-257.

19. Longnecker, S.M.; Donehower, R.C.; Cates, A.E.; Chen, T-L.; Brundrett, R.B.; Grochow, L.B.; Ettinger, D.S.; Colvin, M. *Cancer Treatment Reports*, **1987**, 71, 53-59.

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