Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 5, No. 3, pp. 247-252, 1995

0960-894X/95 $9.50+0.00

0960-894X(95)00018-6

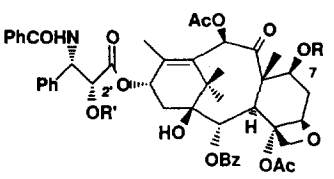
NOVEL, WATER-SOLUBLE PHOSPHATE DERIVATIVES OF 2'-ETHOXY CARBONYLPACLITAXEL AS POTENTIAL PRODRUGS OF PACLITAXEL: SYNTHESIS AND ANTITUMOR EVALUATION

Yasutsugu UEDA,* John D. MATISKELLA, Amarendra B. MIKKILINENI†, Vittorio FARINA‡, Jay O. KNIPE, William C. ROSE†, Anna Maria CASAZZA†, and Dolatrai M. VYAS

Bristol-Myers Squibb Company, Pharmaceutical Research Institute
5 Research Parkway, Wallingford, CT 06492-7660

Abstract: Three new phosphate derivatives of paclitaxel-2'-ethylcarbonate **4a**, **4b**, and **4c**, have been synthesized and evaluated for *in vivo* antitumor activity. All were soluble in water and two derivatives **4a** and **4b** were found to exhibit *in vivo* antitumor activity, comparable to paclitaxel in the M109 murine tumor model.

It is well recognized that one of the major problems associated with paclitaxel is its poor water-solubility. In our previous report,[1] we described a unique finding that paclitaxel-2'-ethylcarbonate **2** exhibited remarkable *in vivo* antitumor activity, comparable if not superior to paclitaxel **1**, acting like a prodrug of paclitaxel. However, it was as poorly soluble in water as paclitaxel, and a search for water-soluble prodrug derivatives was therefore initiated. Recently, we demonstrated that paclitaxel derivatives having a phosphonoxyphenylpropionate ester group at the 2'-position **3a** or at the 7-position **3b** had much improved water-solubility over paclitaxel and also liberated paclitaxel upon exposure to alkaline phosphatase, proving them as potentially useful water-soluble prodrugs.[2]

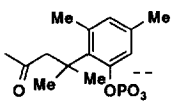


1; R = R' = H, paclitaxel

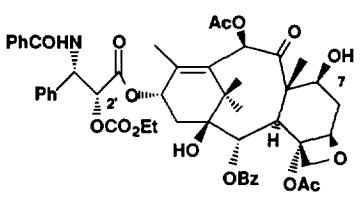
2; R = H, R' = CO_2Et, paclitaxel-2'-ethylcarbonate

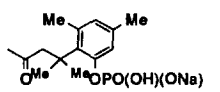
3a; R = H, R' = LA

3b; R = LA, R' = H

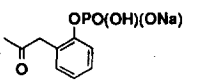
LA = 

To improve water-solublity of 2'-ethoxycarbonylpaclitaxel **2**, we investigated the introduction of highly ionizable phosphate moieties into the 7-position of this molecule. Here we describe the synthesis and *in vivo* antitumor activity of paclitaxel-2'-ethylcarbonates **4a**, **4b** and **4c** having three different phosphate linkers at the 7-position .



4a; R = 

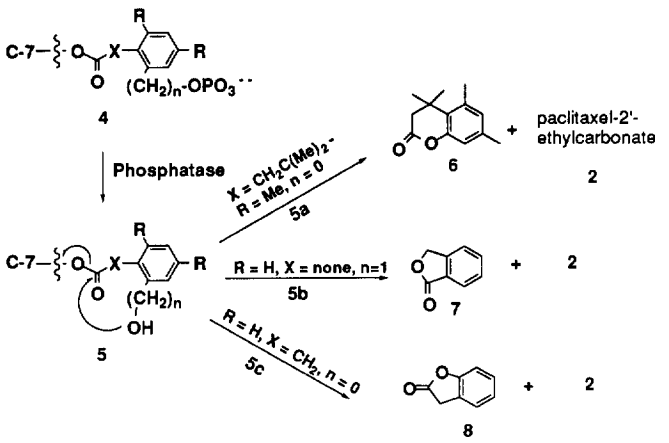
4b; R = 

4c; R = 

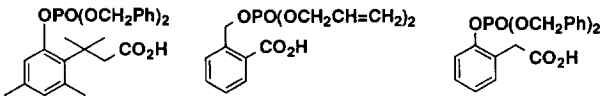
Along with phosphonoxyphenylpropionate ester **4a**, we have also investigated the simplified phosphate-linked esters **4b** and **4c** which carry a non-methylated phosphate-phenyl moiety at C-7. As discussed in our earlier publication,[2] the phosphate moiety in **4a** should serve not only as a water solubilizing function, but also as a site of the substrate for alkaline phosphatases, common enzymes in mammalian systems, which hydrolyze the phosphate **4a** to the hydroxy ester **5a**. This ester **5a** then, lactonizes rapidly with assistance of "trimethyl lock" acceleration[3] to generate the parent compound, paclitaxel-2'-ethylcarbonate **2** as illustrated in Scheme 1.

The phosphate esters **4b** and **4c** were designed similarly to generate paclitaxel-2'-ethylcarbonate in a two-step process as shown in Scheme 1. Upon exposure to alkaline phosphatases, they should liberate the intermediate hydroxy esters **5b** and **5c**, which then should cyclize[4] to γ-lactones, phthalide (**7**) and 2-coumaranone (**8**), respectively, generating paclitaxel-2'-ethylcarbonate **2**. Hydronium ion-catalyzed lactonization of the corresponding hydroxy acids to γ-lactones **7**[4b] and **8**,[4c] and in particular the lactonization of 2-hydroxymethylbenzoic acid to phthalide (**7**) is reported to be very rapid.[4b] Although the use of 2-hydroxymethylbenzamide as a potential prodrug substituent of amine-containing molecules has previously been documented,[4d] phosphatase-assisted γ-lactone formation with liberation of the parent drug is unprecedented.

Scheme 1



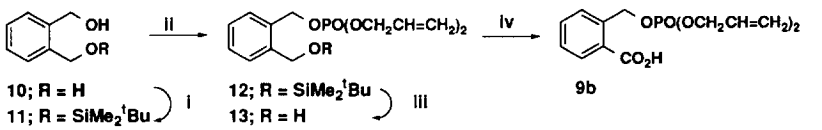
The preparation of the requisite linker acid **9a** was described in our recent paper.[2] The other simplified phosphate linker acids, *o*-phosphonoxymethylbenzoic acid **9b** and *o*-phosphonoxyphenylacetic acid **9c** were prepared by similar methods from 1,2-di(hydroxymethyl)benzene (**10**) and 2-hydroxyethylphenol **14** as illustrated in Schemes 2 and 3, respectively.



Unlike the phosphonoxyphenylpropionate ester **4a**, the phosphonoxymethylbenzoate ester **4b** possesses a benzyl phosphate moiety. This is a potential problem during hydrogenolysis if the benzyl group is used as a phosphate protection as applied for the preparation of the linker acid **9a**. For this reason, we chose the allyl group as the phosphate protecting group of the linker acid as seen in **9b**.

Monosilyl alcohol **11**[5] separated in 40% yield from silylation of **10**, was phosphorylated by treatment with bis(allyl)(diisopropylamino)phosphine,[6] followed by oxidation with *m*-CPBA to give diallyl phosphate **12**[7] in 77% yield. The silyl group was cleaved by aqueous HCl in isopropanol to provide phosphonoxymethylbenzyl alcohol **13**[7] which was oxidized with Jones reagent[8] to the target linker acid **9b**[7] in overall yield of 88%.

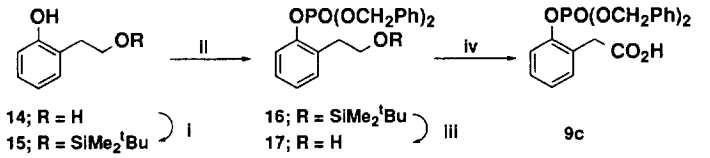
Scheme 2



Reagents and Conditions:
i) $ClSiMe_2{}^tBu$/Im/DMF, rt, 18h (y. 40%), ii) a) $(i\text{-}Pr)_2NP(OCH_2CH{=}CH_2)_2$, tetrazole/$CH_2Cl_2$, rt, 4h, b) *m*-CPBA/$CH_2Cl_2$, -40° - 5°C, 1h (y. 77%), iii) aq. HCl/i-PrOH, rt, 3h (y. 89%), iv) Jones reagent/acetone, rt, 1/2h (y. 98%).

The third linker acid, dibenzylphosphonoxyphenylacetic acid **9c** was prepared similarly from hydroxyphenylethanol **14** in 4 steps as shown in Scheme 3. The phosphorylation of silyloxyethylphenol **15**, which was prepared by selective silylation of hydroxyphenol **14**, by treatment with n-BuLi in THF/hexane, followed by reaction with tetrabenzyl pyrophosphate[9] furnished phosphate triester **16**[7] in quantitative yield. The removal of the silyl protecting group with aqueous HCl in isopropanol, followed by Jones oxidation[8] produced the linker acid **9c**[7] as white crystals in 57% yield.

Scheme 3

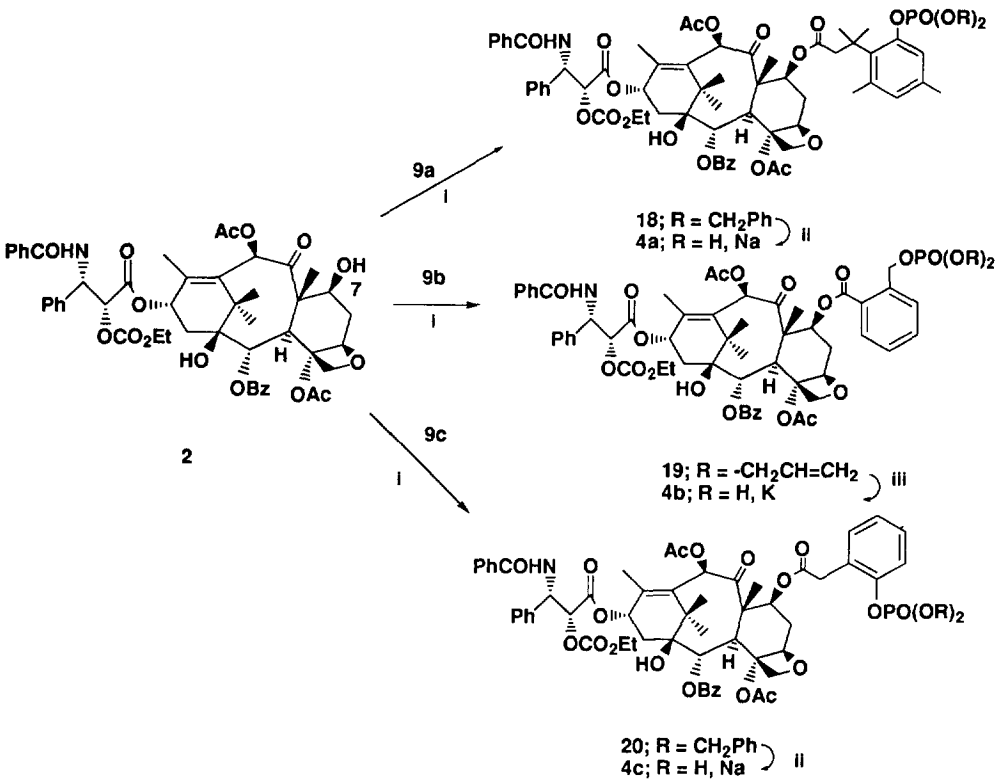


Reagents and Conditions:
i) $ClSiMe_2{}^tBu$/Im/DMF, rt, 3h (y. 100%), ii) a) n-BuLi/Hex-THF, 0-5°C, b) $[(PhCH_2O)_2PO]_2PO]_2O$, rt, 2h, (y. 100%), iii) aq. HCl/i-PrOH, rt, 1h (y. 100%), iv) Jones reagent/acetone, rt, 45 min (y. 57%).

These three linker acids **9a**, **9b**, and **9c** were introduced at the 7-position of paclitaxel-2'-ethylcarbonate **2** as illustrated in Scheme 4. Thus, acylation of 2'-ethylcarbonate **2**[1] with linker acids **9a**, **9b**, and **9c** (1.75 - 2

eq.) using 1,3-dicyclohexylcarbodiimide (DCC, 1.9 - 4 eq.) and 4-dimethylaminopyridine (DMAP) as activating agents[10] produced 7-acylpaclitaxel-2'-ethylcarbonates **18**,[7] **19**,[7] and **20**,[7] respectively. It was noted that acylation with benzoic acid **9b** and phenylacetic acid **9c** was substantially faster than that with phenylpropionic acid **9a**. The benzyl protecting groups in **18** and **20** were cleaved by catalytic hydrogenation to furnish the target phosphonoxyphenylalkanoates **4a**[7] and **4c**,[7] respectively as their mono-sodium salts after treatment with sodium bicarbonate followed by purification using C-18 reverse phase chromatography. The removal of the diallyl protecting group in **19** was achieved by Pd (0) catalyzed reaction in the presence of $Bu_3SnH/HOAc$,[11] yielding the desired 7-phosphonoxymethylbenzoate **4b**[7] as its mono-potassium salt after treatment with potassium 2-ethylhexanoate and potassium bicarbonate followed by C-18 reverse phase column purification.

Scheme 4



Reagents and Conditions:
i) dicyclohexylcarbodiimide (DCC) (1.9 - 4 eq.)/dimethylaminopyridine (DMAP) (0.5 - 1.4 eq.) /CH_2Cl_2, rt; **9a** (1.75 eq.), 3 days (y. 55%); **9b** (2 eq.), 15h (y. 71%); **9c** (2 eq.), 18 h (y. 77%) ii) a) H_2/Pd-C/EtOH-EtOAc, 40 psi, 3-5h; b) $NaHCO_3/H_2O$-CH_3CN, C-18 reverse phase column (y. 54% for **4a**; y. 36% for **4c**) iii) a) $Pd(PPh_3)_4$-HOAc-Bu_3SnH/CH_2Cl_2, rt, 5 days, b) potassium 2-ethylhexanoate/ $KHCO_3/CH_2Cl_2$-EtOAc, then C-18 reverse phase column (y. 34%).

These paclitaxel phosphate derivatives **4a**, **4b**, and **4c** were found to be much more soluble in water (2.5-5 mg/mL H_2O) than paclitaxel. Compounds **4a** and **4b** were also stable at pH 7.4, T_{90} being over 150 hrs for **4a** and 60 hrs for **4b** at 37°C.[12]

As designed, water-soluble phosphonoxyphenylpropionate derivatives **4a** and **4b** generated paclitaxel-2'-ethylcarbonate **2** rapidly ($T_{1/2}$[13] of less than 5 min.) in contact with alkaline phosphatase (Sigma, obtained from bovine intestinal mucosa, incubated at 37°C, pH 7.4 or 7.8).

The *in vivo* antitumor activity of these water-soluble phosphate derivatives **4a-4c** is summarized in Table 1. The phosphonoxyphenylpropionate ester **4a** and the phosphonoxymethylbenzoate derivative **4b** were found to exhibit comparable *in vivo* antitumor activity relative to paclitaxel in this model. However, the last derivative, **4c** was inactive. It was speculated that paclitaxel or paclitaxel-2'-ethylcarbonate was not generated under the conditions. In a 2'-hydroxy series, it was found that alkaline phosphatase cleaved the phosphate moiety of 7-(*o*-phosphonoxyphenylacetyl)paclitaxel to generate the 7-(*o*-hydroxyphenylacetyl)paclitaxel but the lactonization was slow and paclitaxel was not liberated instantaneously ($T_{1/2}$[13] > 200 h). This paclitaxel-7-phosphonoxy phenylacetate was devoid of *in vivo* antitumor activity.[14] Therefore, we believe, in a 2'-ethylcarbonate series, the phosphate ester **4c** also liberated the intermediate hydroxy ester **5c** in contact with alkaline phosphatase but the lactonization was too slow to generate the parent paclitaxel-2'-ethylcarbonate **2**. The inability to generate paclitaxel (**1**) or **2** from 7-(*o*-phosphonxyphenylacetyl)paclitaxel derivatives (e.g. **4c**) spontaneously in contact with alkaline phosphatase may be due to a combination of steric congestion around the C-7 ester which could retard the lactonization and insufficient rate enhancement in lactonization of *o*-hydroxyphenylacetate to 2-coumaranone (**8**).

These results indicated that **4a** and **4b** proved to be useful prodrugs of paclitaxel-2'-ethylcarbonate but **4c** was too stable to be an effective prodrug of paclitaxel-2'-ethylcarbonate.

Table 1: *In vivo* Antitumor Activity of Phosphate Derivatives of Paclitaxel-2'-Ethylcarbonates 4a, 4b, and 4c in M109 Tumor Model

Compound	*In vivo* Antitumor Activity[a] (T-C) Days/Optimal Dose (mg/Kg/Injection)	
	Compound	Paclitaxel
4a	18.0/(40)	12.3/(18)
4b	17.0/(40)	10.8/(24)
4c	1.5/(33)	15.8/(48)

[a]The Madison 109 murine lung carcinoma (M109) s.c. (subcutaneous) implant model was used. Drugs were administered intravenously in water. Five consecutive daily treatment schedule begins on day 4 post-tumor implant. T-C refers to delay in tumor growth, measured in relative median time (days) for tumors to reach 1 gm in drug treated as compared to control groups (8 mice per dose). A drug producing a T-C value of $\geq$ 4 days is considered to be active in this tumor model. T-C values at maximum tolerated dose are listed.

In summary, we prepared novel phosphate derivatives of 2'-ethoxycarbonylpaclitaxel **4a, 4b** and **4c** as potential prodrugs of paclitaxel. All were found to be soluble in water (2.5 - 5 mg/mL) and two derivatives **4a** and **4b** generated paclitaxel-2'-ethylcarbonate **2** upon exposure to the alkaline phosphatase and also exhibited

remarkable *in vivo* antitumor activity, comparable to paclitaxel in the s.c. M109 murine tumor model, proving themselves as useful prodrugs in this animal model.

Acknowledgements: We would like to thank Dr. T.W. Doyle for constructive guidance and our Analytical Research Staff members for spectroscopic and analytical measurements.

References and Notes:

† Present address: Bristol-Myers Squibb Pharmaceutical Research Institute, Princeton, NJ 08543.

‡ Present address: Boehringer-Ingelheim Pharmaceuticals Inc., Ridgefield, CT 06877.

1. Ueda, Y.; Wong, H.; Matiskella, J.D.; Mikkilineni, A.B.; Farina, V.; Fairchild C.; Rose, W.C.; Mamber, S.W.; Long, B.H.; Kerns, E.H.; Casazza, A.M.; Vyas, D.M. *BioMed. Chem. Lett.* **1994**, *4*, 1861.

2. Ueda, Y.; Mikkilineni, A.B.; Knipe, J.O.; Rose, W.C.; Casazza, A.M.; Vyas, D.M. *BioMed. Chem. Lett.* **1993**, *3*, 1761.

3. Amsberry, K.L.; Gerstenberger, A.E.; Borchardt, R.T. *Pharma. Res.* **1991**, *8*, 455 and references therein.

4. a) Prodrugs based on masked lactones: See Johnson, C.D.; Lane, S.; Edwards, P.N.; Taylor, P.J. *J. Org. Chem.* **1988**, *53*, 5130 and references therein.
   b) Fife, T.H.; Benjamin, B.M. *J. Amer. Chem. Soc.* **1973**, *95*, 2059.
   c) Hillery, P.S.; Cohen, L.A. *J. Org. Chem.*, **1983**, *48*, 3465.
   d) Cain, B.F.; *J. Org. Chem.* **1976**, *41*, 2049.

5. Anderson, K.A.; Kinder, F.R.Jr. *J. Heterocyclic Chem.* **1990**, *27*, 975.

6. a) Banwarth, W.; Kung, E. *Tetrahedron Lett.* **1989**, *30*, 4219.

   b) Yu, K.-L.; Fraser-Reid, B. *Tetrahedron Lett.* **1988**, *29*, 979.

7. All new compounds gave analytical and spectroscopic results consistent with the assigned structure.

8. Fieser, L.F.; Fieser, M. *Reagents for Organic Synthesis*; John Wiley and Sons, Inc.: New York, **1967**, Vol. 1, p. 142.

9. a) Watanabe, Y.; Nakahira, H.; Bunya, M.; Ozaki, S. *Tetrahedron Lett.* **1987**, *28*, 4179.
   b) Ley, S.V.; Parra, M.; Redgrave, A.J.; Sternfeld, F. *Tetrahedron Lett.* **1990**, *46*, 4995.

10. Mathew, A.E.; Mejillano, M.R.; Nath, J.P.; Hines, R.H.; Stella, V.J. *J. Med. Chem.* **1992**, *35*, 145.

11. Dangles, O.; Guibe, F.; Balavoine, G. *J. Org. Chem.* **1987**, *52*, 4984.

12. The solubility in deionized water was determined by HPLC after filtration through 0.2 μm Nylon filters. The stability was measured in 50 mM tris buffer and the T_{90} is defined as the time required for 90 % of the initial compound to be intact. The T_{90} for compound **4c** was not determined.

13. The $T_{1/2}$ is defined as the time required to generate 50 % of the parent drug with cleavage of the linker moiety. The $T_{1/2}$ for compound **4c** was not determined, but it was estimated to be > 200 h, based on the $T_{1/2}$ for the corresponding 2'-hydroxy analog.

14. The details will be reported in a full account.

(*Received in USA* 25 *November* 1994; *accepted* 3 *January* 1995)