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NOVEL WATER SOLUBLE PHOSPHATE PRODRUGS OF TAXOL® POSSESSING IN VIVO ANTITUMOR ACTIVITY§

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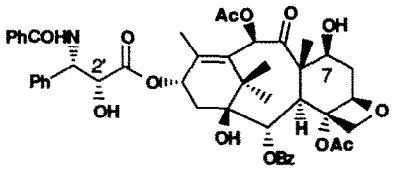
Abstract: Synthesis and biological evaluation of novel taxol derivatives having a phosphonoxyphenylpropionate ester group at the 2'-position or at the 7-position of taxol are described. These were found to have much improved water solubility and both were found to generate taxol upon exposure to alkaline phosphatase. A particular derivative, 7-phosphonoxyphenylpropionate of taxol **3b** exhibited antitumor activity comparable to that of taxol against the ip/ip M109 murine tumor model.

Taxol (**1**), a diterpene natural product, was isolated in 1971 from the western yew[1] and has been shown to be highly cytotoxic and possess potent antileukemic and tumor inhibitory properties.[1,2] Its mechanism of action is unique. Taxol acts by promoting the assembly of stable microtubules from tubulin and to inhibit the disassembly process.[3] Recently taxol has been approved by FDA for the treatment of advanced ovarian cancer, and current clinical studies have shown a remarkable efficacy against other forms of cancer such as melanoma, breast and lung cancer.[4,5]

Although taxol has demonstrated a promise as a unique antitumor agent, it has several drawbacks. One of the major problems is its poor solubility in water, creating formulation difficulty for the intravenous administration. Cremophore EL (polyethoxylated castor oil) and ethanol are presently used as solubilizing agents.[6] However, Cremophore is considered to be partly responsible for some of the adverse effects during the taxol treatment.[5] One approach to solve the solubility problem is to introduce a water soluble substituent which can be readily removed in the body by enzymes to generate the parent taxol (prodrug approach[7]).

A number of groups have recently reported the synthesis and biological evaluation of water soluble prodrugs of taxol. These derivatives have a polar substituent as an ester either at the C-2' hydroxy or at the C-7 hydroxy group.[8] More recently, a phosphate group was introduced in our laboratories at the C-2' and also at the C-7 position of taxol,[9] since the phosphate of etoposide was effective as a prodrug.[10] These taxol phosphates were more water soluble than taxol, but in vitro they were stable both in plasma and towards alkaline phosphatases[11] and in vivo exhibited no efficacy. It was thought that the attachment of the anionic phosphate moiety was too close to the taxol nucleus to be accepted as a substrate for the alkaline phophatases. Thus, for a phophatase-strategy to succeed, it appeared essential to design a substrate which allows ready accessibility to the enzyme.



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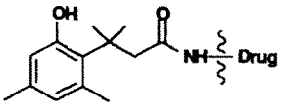

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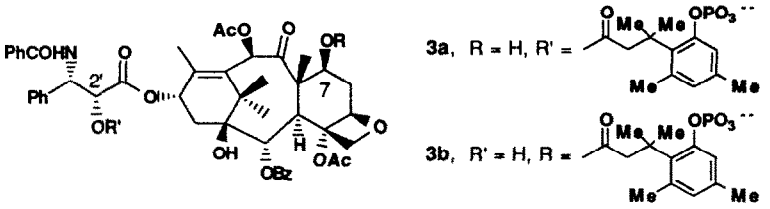
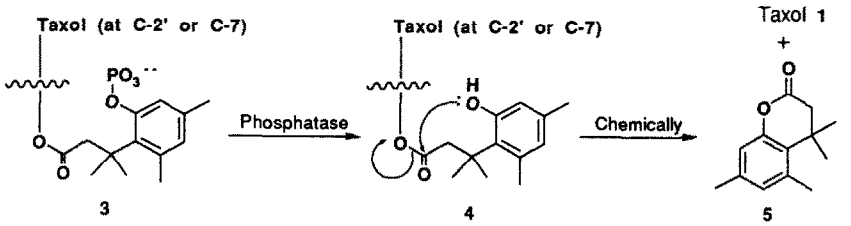
Use of highly methylated 2'-hydroxyphenylpropionic acid amide **2** has been reported as a potential prodrug for amines.[12] We thought the phosphonoxyphenylpropionic acid esters of taxol **3a** and **3b** might fulfill the requirement for a water soluble prodrug of taxol. These phosphonoxyphenylpropionyltaxols are designed to exhibit improved water solubility by having a highly ionizable phosphate group. The phosphate portion of these new taxol derivatives would serve as a moiety readily recognized and cleaved by enzymes called phosphatases, more specifically alkaline phosphatases whose distribution is ubiquitous in biological systems.[11] As the phosphate moieties are located away from the taxol nucleus, these should be sterically more accessible to the enzyme than the taxol phosphates reported earlier.[9]



Once the phophatase cleaves the phosphate moiety of prodrugs **3**, the hydroxyphenylpropionate ester **4**, with assistance of "trimethyl lock" acceleration,[12,13] should rapidly lactonize to generate taxol and hydrocoumarin **5**, as illustrated in Scheme-1. The C-7 ester **3b** is of particular interest to us. Taxol derivatives having an ester group at the C-7 position have been shown to be less susceptible than the corresponding C-2' ester to enzymatic cleavage to taxol.[8d] However, the C-7 ester **3b** is specifically designed to generate the parent taxol in vivo, not by enzymatic hydrolysis of the carboxylate but by phosphatase-initiated lactonization.

Scheme-1

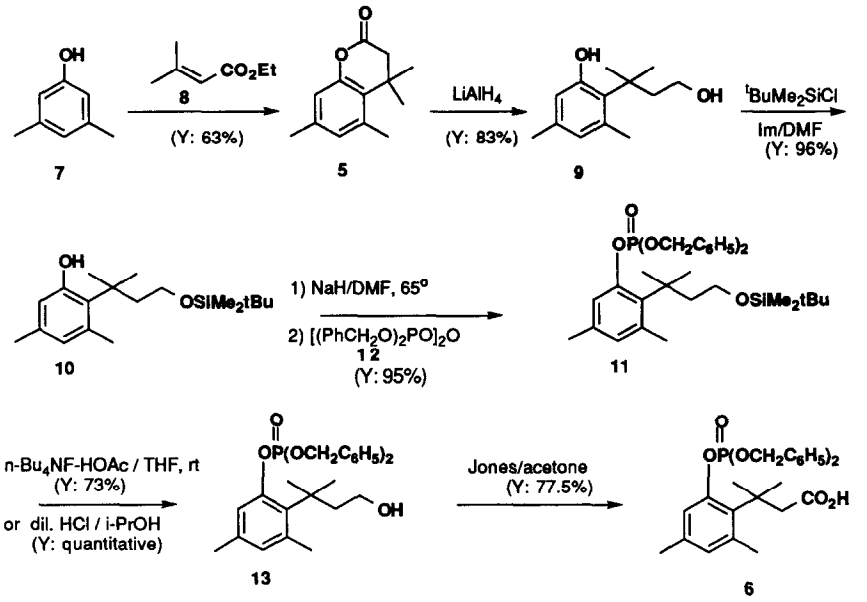


3 4 5

This mechanism of generating the parent taxol, assisted by phosphatases followed by rapid lactonization is unique and unprecedented.

Here, we report the synthesis and biological evaluation of novel water soluble phosphonoxyphenyl propionate esters of taxol **3a** and **3b**. The requisite linker, diphenylphosphonoxyphenylpropionic acid **6** was prepared from 3,5-dimethylphenol **7** as shown in Scheme-2.

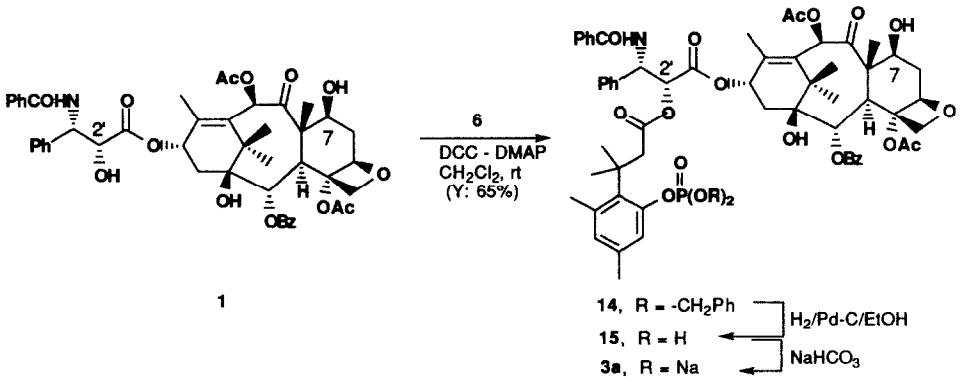
Scheme-2



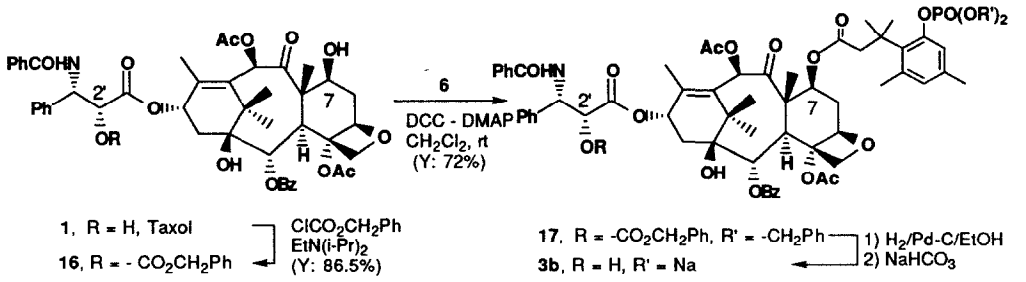
The dibenzylphosphate moiety was previously used as a phosphate protecting group in the synthesis of etoposide phosphate[10] and it was hydrogenolytically cleaved to produce a water soluble anionic phosphate functionality. The diol **9** was prepared using a literature procedure[12] from phenol **7** via hydrocoumarin **5** in about 52% yield.[14] The primary hydroxy group of diol **9** was silylated to siloxypropylphenol **10**[12b] in 96% yield. Phosphorylation[15] of this phenol **10** using diphenyl chlorophosphate[10a] and diisopropylethylamine was not successful. The phosphate triester **11**[16] was obtained in 95% yield by generating the phenoxide either with NaH in DMF at 65°C and then treating with pyrophosphate **12**.[17] The silyl protecting group was removed with tetrabutylammonium fluoride (TBAF) and acetic acid in THF or more conveniently by treatment with dilute acid in i-PrOH to furnish hydroxy-phosphate **13**[18] in high yield. The primary alcohol in **13** was oxidized by treatment with the Jones reagent in acetone to provide the desired acid **6** in 77.5% yield.

It has been well documented[19] that the C-2' hydroxy group of taxol is more readily acylated than the C-7 hydroxyl. Acylation of taxol (**1**) with 1 eq. of phosphonoxyphenylpropionic acid **6** using 1,3-dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (DMAP) as activating agents[8d] cleanly gave the C-2' phosphonoxyphenylpropionate ester **14** in 65% yield. Formation of the 2',7-bisester was observed when more than 1 eq. of the acid **6** was employed. The benzyl groups in **14** were cleaved by catalytic hydrogenation

(H_2/Pd-C) to furnish the C-2' phosphoric acid **15** which was treated with 2 eq. of $NaHCO_3$ in H_2O, giving the target 2'-ester phosphate derivative of taxol **3a** as sodium salt in 78% yield, after column purification on C-18 reverse phase silica gel.



For the synthesis of the C-7 ester **3b**, the C-2' hydroxy group was first protected as its benzyl carbonate **16**. Acylation of this carbonate at the C-7 position was much slower but achieved using excess acid **6** and excess DCC, producing the C-7 phosphonoxyphenylpropionate ester **17** in 72% yield. Catalytic hydrogenation as above removed the benzyl groups on the phosphate moiety as well as on the benzylcarbonate protecting group at the C-2' position, furnishing the target molecule, 7-ester phosphate derivative of taxol **3b** as sodium salt in 71% yield, after treatment with 2 eq. of $NaHCO_3$ and purification by column chromatography using C-18 reverse phase silica gel.



The sodium salts of taxol phosphate, **3a** and **3b** were found to be much more soluble in water (>10 mg/mL H_2O) than taxol. Both derivatives were also stable in water. No apparent decomposition was observed in deionized water at room temperature in the concentration of 8 mg/mL for 24 hrs. 2'-Phosphonoxyphenylpropionyl derivative **3a** appeared to be less stable than the corresponding 7-acyl derivative **3b**.[20] As designed originally, these new water-soluble taxol derivatives were found to be readily cleaved to generate taxol (>90% in 25 min at 37°C) in the presence of purified alkaline phosphatase preparations (bovine intestinal mucosa, bovine liver, human placenta) at pH 7.4, fulfilling one of the requirements as prodrugs. However when these derivatives were

incubated with rat or dog plasma, no sign of taxol liberation (hplc analysis) was observed even after a 24 hr incubation period. In human plasma, a trace of taxol was detected after 24 hrs. It was also found that taxol generation from these new derivatives with the phosphatase was retarded as added albumin increased, suggesting that these taxol derivatives possibly bind to plasma proteins tightly,[21] making them inaccessible to the phosphatase. These compounds exhibited no activity in the tubulin polymerization assay,[3a] but after incubation with 10 % alkaline phosphatase (bovine intestinal mucosa) at 37°C for 30 min, these derivatives were found to be active in promoting microtubule assembly, indicating again the facile generation of the parent drug taxol in contact with the alkaline phosphatase.

These water-soluble taxol phosphates **3a** and **3b** were evaluated in vivo against the murine solid tumor (M109) model.[22] The in vivo antitumor results are summarized in Table-1. These results indicated that the 2'-phosphonoxyphenylpropionyltaxol **3a** was marginally active at its optimal dose, 100 mg/Kg/inj., (T/C = 144 %) compared with taxol (T/C = 275 %). However, using a slightly different treatment protocol, we were delighted to find that the corresponding C-7 acyl taxol **3b** was as efficacious as taxol at its maximum tolerated dose in this in vivo evaluation model system, T/C being 156 % favorably compared with that of taxol, T/C = 144%.

In summary, we have succeeded the synthesis of water-soluble novel taxol derivatives **3a** and **3b**, and have demonstrated that these water-soluble taxol phosphates are readily cleaved to liberate the parent taxol in contact with the phosphatase. Most interestingly, 7-phosphonoxyphenylpropionyl derivative of taxol **3b** was found to exhibit comparable activity to taxol in the murine M109 tumor model.

Table-1 In Vivo Antitumor Activity of Compounds 3a and 3b in M109 Tumor Model[a]

			% T/C[b] (mg/Kg/Injection)[c,d]	
Experiment	Compound	Vehicle	Compound	Taxol[e]
1[c]	**3a**	water	144 % (100)	275 % (30)
2[d]	**3b**	water	156 % (140)	144 % (40)
			153 % (70)	144 % (40)

a, Murine lung carcinoma, i.p.(intraperitoneal) implant model.

b, T/C refers to the percentage of the median survival time of drug-treated mice (six per dose) to saline-treated controls.

c, Dose administered i.p. on days 1,5 and 9.

d, Dose administered i.p. on days 5 and 8.

e, Administered in 10 % Tween 80 in saline.

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§ Taxol® is a registered trademark of Bristol-Myers Squibb Company.
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