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SYNTHESIS AND ANTITUMOR EVALUATION OF WATER SOLUBLE TAXOL PHOSPHATES

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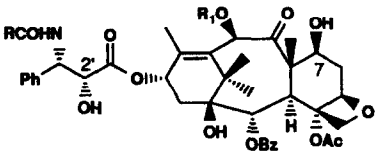
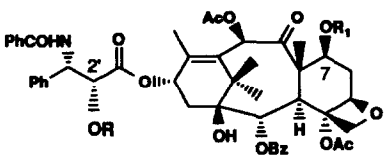
ABSTRACT: *Synthesis and in vivo antitumor evaluation of water soluble 2'- and 7- taxol phosphates is reported. These derivatives were found to be poor prodrugs of taxol based on their marginal in vivo antitumor activity against ip,ip M109 murine tumor model and taxol release studies in vitro.*

Taxol (**1**)[1], based on its clinically observed broad spectrum antitumor activity[2] against human solid tumors such as melanoma, ovary, breast and lung, has been heralded as the anticancer drug of the 1990's. Currently, taxol is an FDA approved drug for the treatment of human ovarian carcinoma refractory to cis-platinum. Taxotere (**2**)[3], a close analog of **1**, is also receiving extensive clinical attention. Both of these agents, however, suffer from serious pharmaceutical liabilities in the clinic in not being readily soluble in a wide variety of acceptable formulation vehicles for intravenous administration. This is attributed to their extreme insolubility in water; taxotere (6-7 μg/ml) being slightly more soluble than taxol (0.25 μg/ml)[4]. Furthermore, some of the side effects such as hypersensitivity reactions (hypotension, bronchospasm, urticaria etc.) observed after taxol administration are considered partly to be formulation (Cremophor EL) related and consequently have impeded rapid and full clinical exploitation of this drug[5].



1. TAXOL R = Ph , R_1 = - $COCH_3$
2. TAXOTERE R = OtBu , R_1 = H



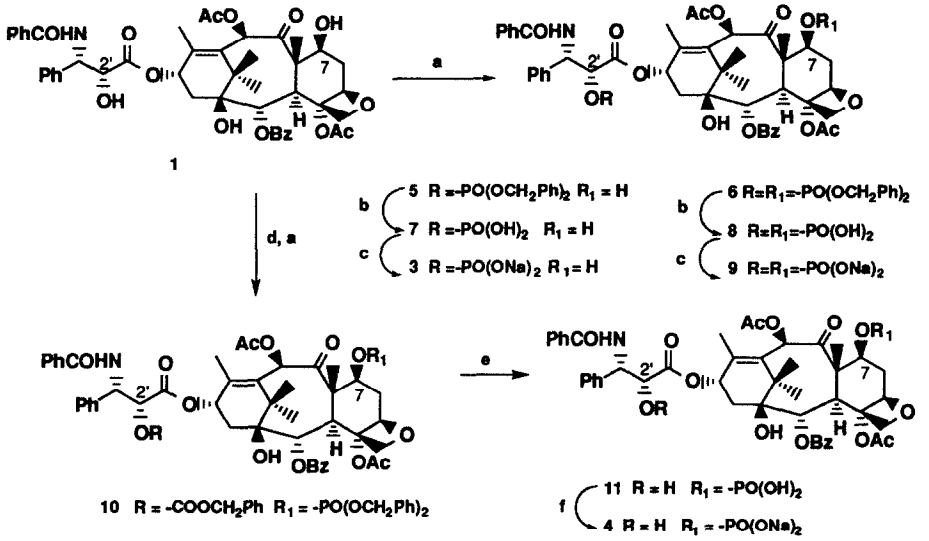
3. R = $PO(ONa)_2$, R_1 = H
4. R = H, R_1 = $PO(ONa)_2$

In addressing the solubility issue, several research groups have reported synthesis and biological results of a variety of water soluble prodrugs of taxol[6]. All strategies reported, involve synthesis of prodrugs with a 2'-ester moiety carrying a water solubilizing functionality; the unmasking of these to taxol in vivo is assumed to be through cleavage by endogenous esterase activity. In contrast, the corresponding C-7 esters[6] were found to be poor prodrugs and analogs due to their lack of interaction with the esterases and poor cellular uptake respectively.

Our laboratory has focused on a different strategy involving synthesis and evaluation of taxol phosphate derivatives **3** and **4** as potential phosphatase cleavable prodrugs. Our previous such attempt in the case of antitumor agent etoposide was highly successful[7]. In this communication we wish to disclose our preliminary findings on this hitherto unreported class of taxol phosphate derivatives.

At the outset several attempts towards synthesis of desired targets **3** and **4** employing standard phosphorylation reactions involving the use of $POCl_3$[8], $(PhO)_2POCl$[9], $(CCl_3CH_2O)_2POCl$[10] met with little success. In using both the phosphochloridate reagents the initial phosphorylation reactions were successful, however, all of the methods reported for removal of the blocking groups failed to yield the desired products. Eventually, reaction employing the pyrophosphate method[11] was found to be useful for the prodrug synthesis purposes. Thus, treatment of taxol dianion generated at -30° C with LDA in THF with dibenzyl pyrophosphate afforded a mixture of monophosphate ester **5** (33%) and diester **6** (61%)[12]. These esters upon hydrogenolysis were converted to their corresponding acids **7** and **8** respectively (Scheme 1). Subsequently, these acids were readily converted for biological evaluations to their corresponding water soluble (>10mg/ml) disodium salts **3** and **9.**

One of our intentions in this program was to prepare and test the C-7 phosphate salt of taxol either as a potential water soluble prodrug or an analog. It is well established that substitution at C-7 can lead to active analogs[6,13]. Since selective phosphorylation of taxol at C-7 OH was not possible by the pyrophosphate



Scheme 1 **Reaction conditions: a: LDA/THF/-30°C, O(PO(OCH$_2$Ph)$_2$)$_2$; b: EtOAc/Pd/C(10%) /H$_2$, 40 psi; c: MeOH/NaOH; d: CH$_2$Cl$_2$/LDA/0°C,ClCOOCH$_2$Ph, e: EtOH/Pd/C(10%) /H$_2$, 45 psi; f: CH$_3$CN/NaOH**

methodology, it had to be converted to its C-2'CBz derivative first before attempting phosphorylation. Accordingly, the water soluble (>10mg/ml) C-7 disodium phosphate salt **4** was prepared from taxol via the 2'-CBz derivative **10** and acid **11** by using the pyrophosphate methodology (scheme 1) discussed above.

Next we proceeded to study the behavior of water soluble derivatives **3, 9** and **4** in the standard in vitro tubulin polymerization assay[14,15]. In this assay all three compounds were found to be inactive in promoting microtubule assembly. In light of literature precedent[6] this behavior was regarded normal for compounds **3** and **9** since the C-2' hydroxyl functionalities are masked. However, the total lack of tubulin activity of **4** is somewhat surprising since C-7 substitution or functionalization is known to retain tubulin activity in most of the cases reported[6,13]. ***To the best of our knowledge this is the first example of an analog carrying an anionic charge at position C-7; analogs with cationic charge at C-7 are known and active in promoting microtubule assembly[6]***. This observation may reflect an unfavorable charge interaction between **4** and tubulin that inhibits the initial assembly process for microtubule formation.

It was hypothesized that **3**, with only the C-2' position masked, could yield taxol via metabolic cleavage of the C-2' phosphate group. To determine the suitability of **3** as a prodrug of taxol, **3** was incubated (37°C, with shaking) with either purified bovine intestinal alkaline phosphatase (# P-7640 from Sigma Chemical Co.) or freshly prepared heparinized rat plasma. After a 4-hour incubation of **3** with alkaline phosphatase at pH 7.4 (enzyme activity verified using para-nitrophenyl phosphate), 98% (hplc determination) of **3** remained intact; extending this time to 24 hours resulted in a further 20% consumption of **3**. During this entire incubation period there was no indication (hplc) of taxol generation. In the rat plasma study **3** was degraded with time (12% loss in 4 hours and 50% loss in 24 hours) but yielded no taxol as determined by hplc analysis. Furthermore, samples of **3** pretreated upto 3 hours with either rat plasma, serum, liver S9 or purified alkaline phosphatase were not active in the tubulin polymerization assay. These results clearly indicate that **3** is not a prodrug of taxol. Compound **4** was also similarly determined not to be a prodrug of taxol.

The water soluble taxol phosphates **3, 9** and **4** were next subjected to in vivo evaluation against a taxol sensitive murine M109 solid tumor model[16]. The antitumor results are summarized in Table 1. Significant activity in this tumor model is defined as a T/C >125%. In each experiment taxol was used as a positive control. Inspection of the data in Table 1 clearly shows that based on the activity criteria, only the C-2' phosphate **3** exhibits anti tumor activity (T/C=140); albeit much inferior to that of taxol (T/C=290). This clearly suggests that **3** is not a suitable prodrug of taxol, a result in conformity with the preceeding in vitro results. This finding is in contrast to the esterase sensitive C-2' ester prodrugs of taxol. The dibenzyl phosphate ester **5** as expected is found inactive due to its stability towards phosphatase cleavage to taxol in-vivo.

In conclusion, our current study has been successful in addressing the formulation issue. However, the aim of utilizing phosphate derivatives **3** and **4** as suitable prodrugs of taxol has not been realized. Our current findings will be of significance for future water soluble prodrug and analog design.

Table 1. Antitumor Activity of Compounds 5, 3, 9 and 4 in M109 Tumor Model[a]

Experiment	Compound	Vehicle	Maximum T/C[b] (mg/kg/Inj)[c]	
			Compound	Taxol[e]
1	**5**	DMSO→saline	107(50)	270(50)[d]
1	**3**	Water	140(25)	270(50)[d]
2	**9**	Saline	103(30)	190(10)
2	**4**	Water	123 (30)	190(10)

[a]Murine lung carcinoma i.p. implant model.
[b]T/C refers to the percent of the median survival time of drug-treated mice (6 per dose) to saline treated controls.
[c]Dose administered i.p. on days 1, 5 and 9.
[d]Also a dose 25 mg/kg/inj achieved a T/C of 240 with 1/6 mice cured.
[e]Administered in 10% Tween 80 in saline.

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