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SYNTHESIS AND ANTITUMOR EVALUATION OF 2'-OXYCARBONYLPACLITAXELS (PACLITAXEL-2'-CARBONATES)

Yasutsugu Ueda*, Henry Wong, John D. Matiskella, Amarendra B. Mikkilineni†, Vittorio Farina‡, Craig Fairchild†, William C. Rose†, Stephen W. Mamber, Byron H. Long†, Edward H. Kerns, Anna Maria Casazza†, and Dolatrai M. Vyas*

Bristol-Myers Squibb Pharmaceutical Research Institute
5 Research Parkway, P.O. Box 5100, Wallingford, Connecticut 06492-7660

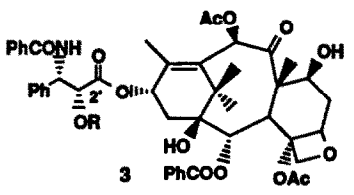
Abstract: A number of 2'-oxycarbonylpaclitaxels (paclitaxel-2'-carbonates) **3** have been prepared and evaluated for their cytotoxicity and *in vivo* antitumor activity. Most of these paclitaxel-2'-carbonates were found to exhibit *in vivo* antitumor activity in the i.p. M109 murine tumor model system, comparable to that of the parent drug.

Taxol® (**1**) (paclitaxel)[1] is regarded as one of the most promising antitumor agents to emerge in the past decade. It has broad spectrum of antitumor activity against several human tumors and a unique mechanism of action, promoting tubulin polymerization and inhibiting the disassembly process.[2] Paclitaxel was recently approved by the FDA for the treatment of refractory ovarian cancer and has also been shown to exhibit promising clinical efficacy against other forms of cancer such as melanoma, breast and lung cancer.[3]

Structure-activity relationship studies[4] have revealed that introduction of an acetyl group at the 2'-position of paclitaxel resulted in loss of activity to promote tubulin polymerization, but not cytotoxicity nor *in vivo* antitumor activity, indicating the 2'-acetate **2a** was readily hydrolyzed back to paclitaxel **1** under a cell culture bioassay and *in vivo* conditions.[5] These observations lead to the design of a number of prodrugs of paclitaxel by introduction of a variety of acyl moieties at the 2'-position. While many of these 2'-acylpaclitaxels (paclitaxel-2'-esters) **2** proved to behave as prodrugs, some suffered from poor chemical stability, limiting their potential.[6]

Some of 2'-oxycarbonylpaclitaxels (paclitaxel-2'-carbonates) such as 2'-trichloroethylcarbonate **3** (R = $-CO_2CH_2CCl_3$) have been used as 2'-protected paclitaxels for further chemical manipulation,[7a] however their antitumor activity has not been reported. The carbonates, in general, are considered to be more stable than the corresponding esters to chemical or enzymatic hydrolysis. It is our assumption that on this basis, the 2'-carbonates of paclitaxel **3** were never investigated for their cytotoxicity or *in vivo* antitumor activity.[7b]

In our program of paclitaxel modification, we have prepared a number of 2'-carbonates of paclitaxel **3a - 3i** and investigated their cytotoxicity against HCT cell line and *in vivo* antitumor activity against the intraperitoneal (i.p.) M109 murine tumor model using i.p. administration of each compound.[8] Herein, we describe the synthesis and antitumor evaluation of these 2'-carbonate analogues of paclitaxel.



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1, paclitaxel: R = H
2, 2'-acylpaclitaxel: R = -COR'
2a, 2'-acetylpaclitaxel: R = $-COCH_3$
3, 2'-oxycarbonylpaclitaxel: R = $-CO_2R'$

The 2'-carbonates **3a - 3i**[9] were prepared[7] from paclitaxel (**1**) and appropriate chloroformates $ClCO_2R'$ **4** (2-4 eq.) using diisopropylethylamine (2-4 eq.) as a base in CH_2Cl_2 as illustrated in the scheme. The reaction conditions and yields of **3** are summarized in Table 1. Under these conditions, the C-2' hydroxyl group was carbonylated selectively as the carbonylation at C-7 was much slower. The 3-(N,N-dimethylamino)phenyl chloroformate **4i** was prepared *in situ* from the corresponding phenol and triphosgene using diisopropyl ethylamine as a base in CH_2Cl_2 at 5°C. Interestingly, the carbonylation of paclitaxel with chloroformate **4i** produced a substantial amount of oxazolone **5**[10] along with the 2'-carbonate **3i**.

Scheme

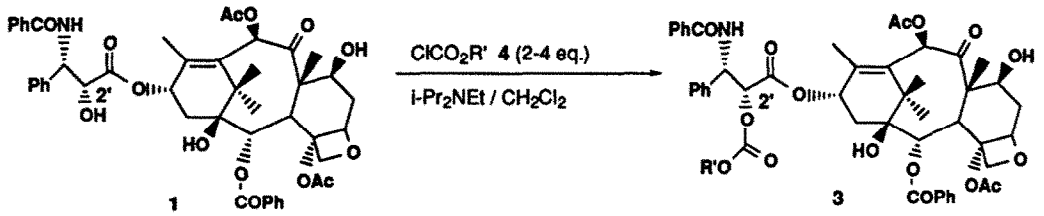

Table 1

Compound	R'	Conditions	% Yields (%)
3a	$-CH_3$	0°, 30 min.	100
3b	$-CH_2CH_3$	0°, 3 h	71
3c	$-CH(CH_3)_2$	rt, 7 days	77
3d	$-CH_2Cl$	0°, 3 h	64
3e	$-CH_2Ph$	rt, 3 h	86
3f	$-CH=CH_2$	0°, 30 min.	100
3g	$-C(CH_3)=CH_2$	0°, 30 min.	75
3h	-Ph	rt, 18 h	64
3i		rt, 5 days	50

The cytotoxicity and *in vivo* antitumor activity are summarized in Table 2. When tested in cytotoxicity assay, paclitaxel-2'-carbonates **3** were 2-10 times less cytotoxic than paclitaxel against human colon cancer cell line (HCT 116). All carbonates were inactive in tubulin polymerization assay. However, after incubation in rat plasma at 37°C for 18 hrs, some of these carbonate derivatives, particularly **3a** and **3b** were found to promote microtubule assembly, indicating the generation of the parent compound, paclitaxel in rat plasma. In the murine

lung carcinoma model M109,[8] all carbonates were effective (%T/C > 125%). The methylcarbonate **3a** was less active than paclitaxel, but all the other carbonates **3b - 3i** exhibited comparable *in vivo* antitumor activity relative to paclitaxel. Apparent superiority of the 2'-ethylcarbonate **3b** (%T/C > 475%) and 2'-vinylcarbonate **3f** (%T/C > 475%) compared to paclitaxel (%T/C = 275%) and most of the other carbonates evaluated was a function of the early initiation of therapy used in that particular experiment and did not represent a significant advantage. In all but one other experiment performed, treatment initiation was delayed until the fifth day post-tumor implant in order to increase the stringency of the antitumor assay.[11]

Table 2: Cytotoxicity and *In Vivo* Antitumor Activity (i.p. M109 Mice Tumor Model) of Paclitaxel-2'-carbonates, 3a - 3i

	Cytotoxicity[a]	*In Vivo* Antitumor Activity[b] %T/C (mg/Kg/Injection)	
Compound	IC_{50}, HCT 116 (μM)	Compound	Paclitaxel
3a	>0.08	162% (90 mg/Kg)[c]	276% (75 mg/Kg)[c]
3b	0.03	>475% (60)[d]	275% (30)[d]
3c	>0.08	247% (100)[c]	197% (50)[c]
3d	0.03	275% (60)[d]	275% (30)[d]
3e	0.03	310% (50)[d]	270% (50)[d]
3f	0.04	>475% (60)[d]	275% (30)[d]
3g	<0.015	235% (80)[c]	262% (75)[c]
3h	0.007	203% (80)[c]	262% (75)[c]
3i	0.016	226% (80)[c]	262% (75)[c]

[a] Cytotoxicity is expressed as IC_{50} against human colon cancer cell line HCT116. IC_{50}: Drug concentration required to inhibit cell proliferation to 50% vs. untreated cells, incubated at 37°C for 72 h. Paclitaxel, IC_{50} (HCT 116) 0.004 μM.

[b] The Madison 109 murine lung carcinoma (M109) i.p. (intraperitoneal) implant model. Drugs administered i.p. in 10% Tween 80 in saline (paclitaxel), in 10% DMSO in saline (**3b - 3f**), or in 10% DMSO in H_2O plus a few drops of Tween 80 (**3a, 3g, 3h,** and **3i**). %T/C refers to the percentage of the median survival time of drug-treated mice (six per dose) to saline-treated control. %T/C at the maximum tolerated dose are listed in the table. %T/C >125% is defined as active in this tumor model.

[c] Dose administered i.p. on days 5, and 8.

[d] Dose administered i.p. on days 1, 5, and 9.

These *in vitro* and *in vivo* results indicate that the paclitaxel-2'-carbonates **3** must have been converted to the parent paclitaxel under the *in vivo* conditions and act like prodrugs of paclitaxel. The *in vitro* drug uptake study using liquid chromatography coupled to tandem mass spectroscopy indicated that incubation of cells with paclitaxel-2'-carbonates, such as **3b** and **3e** resulted in rapid accumulation of paclitaxel and the carbonates in HCT 116 human colon carcinoma cells. This result clearly demonstrates metabolic conversion of 2'-carbonates **3** to the parent paclitaxel. The favorable *in vivo* efficacy observed with most of the paclitaxel-2'-carbonates may

be a partial reflection of their unique distribution and pharmacology *in vivo* system. Such cases are well documented in medicinal chemistry of antitumor agents (e.g., CC-1065, camptothecin).[12]

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References and Notes:

† Present Address: Bristol-Myers Squibb Pharmaceutical Research Institute, Princeton, NJ 08543
‡ Present Address: Department of Medicinal Chemistry, Boehringer-Ingelheim Pharmaceuticals, Inc., 900 Ridgebury Road, Ridgefield, CT 06877.
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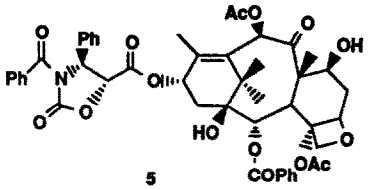
9. All new compounds gave satisfactory analytical and spectroscopic results in accord with the assigned structure.
10. Formation of oxazolone **5**, presumably produced by intramolecular cyclization of **3i**, was not apparant in other cases. Isolation of oxazolone **5** as a major product has been reported from the reaction of trichloroethylcarbonate of paclitaxel with DBU.[4a]



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11. The evaluation of compounds involved two different schedules of treatment, either i.p. injections given on days 1, 5 and 9 post-tumor implant or i.p. injections given on days 5 and 8 post-tumor implant. The latter schedule was adopted in order to increase the stringency of the assay. %T/C values for paclitaxel varied depending on each experiment and the treatment conditions. The *in vivo* activity of paclitaxel-2'-carbonates **3** was evaluated using paclitaxel as a standard in each experiment. All compounds were evaluated at several doses designed to encompass their likely maximum tolerated levels.
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