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Novel C-4 Paclitaxel (Taxol®) Analogs: Potent Antitumor Agents

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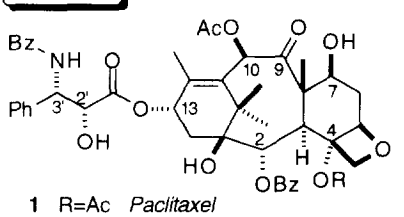
Abstract: A large number of C-4 paclitaxel analogs have been prepared in the course of our systematic C-4 modification. These include C-4 esters, carbonates, carbamates as well as a C-4 deacetyl derivative. All of these analogs were evaluated in a tubulin polymerization assay as well as in a cytotoxicity assay against a human colon cancer cell line. The potent analogs emerging from these in vitro assays were further evaluated in vivo. With the exception of paclitaxel side chain bearing C-4 carbamates and C-4 aromatic esters, most of the C-4 aliphatic esters and carbonates were found to possess comparable or superior activity to paclitaxel in vitro. Several C-4 aliphatic esters and carbonates also exhibited in vivo activities against i.p. implanted murine M-109 lung carcinoma.

INTRODUCTION

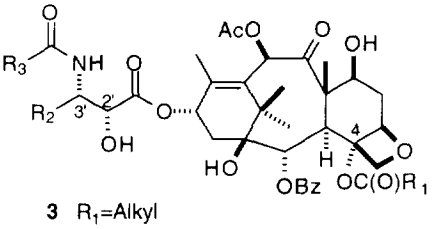
Paclitaxel, **1**, an antimicrotubule agent initially isolated from the bark of *Taxus brevifolia*,[1] has garnered considerable attention in recent years due to its efficacy in the treatment of various types of cancer including ovarian, breast and lung carcinoma.[2] Paclitaxel has a unique mechanism of action which distinguishes it from other anticancer agents. The cytotoxic effects of **1** are believed to arise from its ability to promote tubulin polymerization and stabilize microtubules thus formed even in the absence of cofactors such as (GTP). A major consequence of this shift in tubulin-microtubule equilibrium is the inhibition of mitosis.[3] The continually expanding therapeutic profile of paclitaxel, coupled with its novel mode of action, have spurred intense research activity on many fronts including Structure-Activity Relationship (SAR) studies.[4] Results emerging from rather extensive SAR studies at the diterpenoid core clearly show that the functional groups at C-7, C-9 and C-10 contribute relatively little to receptor binding, whereas, functionalities at C-2 and C-4 together with oxetane ring are essential binding elements.[4]

Figure 1



1 R=Ac *Paclitaxel*

2 R=H *4-Deacetylpaclitaxel*



3 R_1=Alkyl

4 R_1=OMe, OEt, OPr-n

5 R_1=aziridine, imidazole, NH-C $_4H_7$-c

Recently, we[5,6] and others[7,8] have disclosed several interesting approachs to C-4 modification. In this connection, a number of novel C-4 analogs have been reported. These include *bioinactive* derivatives such as the 4-deacetylpaclitaxel,[7] the 4-deacetoxylpaclitaxel,[7] the 4-benzoyl analog[5] as well as several *bioactive* C-4 analogs such as the 4-isobutyl derivative,[8] the 4-cyclopropyl ester[5] and its isosteric 4-aziridine carbamate.[6] In this paper, we report the syntheses and biological evaluation of a large array of novel C-4 derivatives including twenty-three C-4 aliphatic/aromatic ester analogs, eight C-4 methyl/ethyl/n-propyl carbonate bearing derivatives and three carbamate derivatives, as shown in Figure 1.

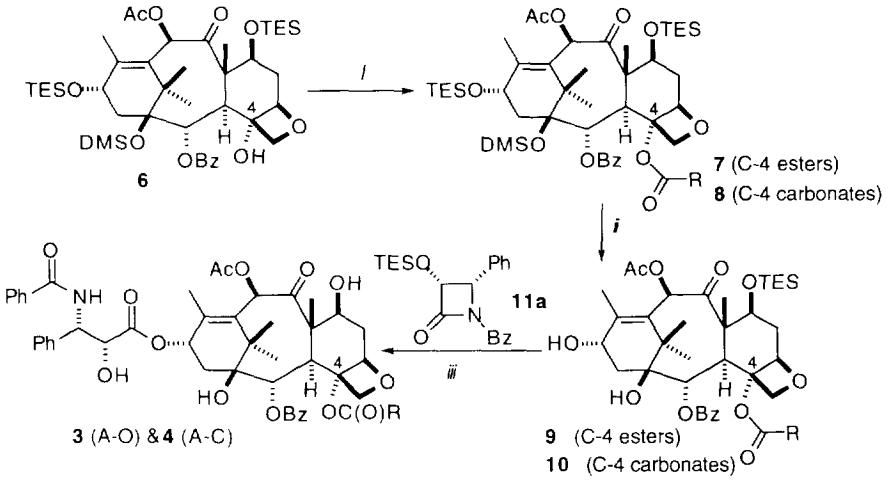
RESULTS AND DISCUSSION

Scheme 1 outlines the synthetic route employed for the syntheses of C-4 ester and carbonate derivatives. As shown in Scheme 1, a total of eighteen paclitaxel side chain carrying C-4 ester/carbonate derivatives, **3**(A-O) and **4**(A-C), were synthesized from 4-deacetyl baccatin **6**, which was prepared in turn by a Red-Al mediated regioselective reduction of the C-4 acetoxyl group using the procedure developed in our laboratory.[5] Deprotonation of 4-deacetyl baccatin derivative **6** with lithium bis(trimethylsilyl)amide, followed by reaction with an acyl chloride or a chloroformate, afforded 42-94% of the 4-ester bearing baccatin **7** or the 4-carbonate carrying baccatin **8**, and then the baccatin derivatives **9** or **10**, after desilylation and C-7 resilylation. Final C-13 side chain attachment onto **9** or **10** was carried out according to the protocol of Holton/Ojima,[9] and provided the desired C-4 ester analogs (**3A-O**) and the C-4 carbonate derivatives (**4A-C**), after final desilylation at C-2' and C-7.
The synthetic scheme for C-4 carbamate derivatives is depicted in Scheme 2. In this case, the C-7 monosilylated 4-paranitrophenyl carbonate baccatin **13** was prepared in three steps from **6** as the key intermediate.[6] Treatment of a THF solution of **13** with cyclobutylamine or imidazole led to the corresponding C-4 carbamate bearing baccatin **15A** or **15B**, respectively. C-13 acylation of **15A/15B** led, after desilylation at C-2 and C-7, to the desired C-4 carbamates **5A** and **5B** in modest yield. The synthesis of C-4 aziridine derivative **5C** was reported recently.[6]

All twenty-one of the C-4 analogs described above were evaluated in a tubulin polymerization assay[10] and an *in vitro* cytotoxicity assay.[11] Eleven of these analogs were also evaluated in a microtubule binding assay[12] (see Table 1). The ratio obtained in the tubulin polymerization assay indicates the potency of analog relative to paclitaxel, and ratios <1 signify analogs as being more potent. The *in vitro* IC_{50} measures the drug concentration required for the inhibition of cell proliferation by 50% after a 72 hours incubation. The cytotoxicity Ana./Tax. IC_{50} ratio is calculated using the IC_{50} values determined for paclitaxel in the same experiment as the analog. The binding assay measures the analog's ability to displace radiolabelled paclitaxel from tubulin and ratios <1 indicate analogs having higher affinity for tubulin than the parent. The results of *in vitro* evaluation of these C-4 analogs are summarized in Table 1. *Careful examination of the data listed in Table 1 reveals that a good correlation existed between the tubulin polymerization assay and the affinity binding assay. It is also clear that results from the cytotoxicity assay are in good agreement with that of the tubulin polymerization assay.*

As shown in Table 1, two C-4 benzoate bearing derivatives (**3A** & **3B**) were considerably less potent than the parent in both the tubulin polymerization assay and the cytotoxicity assay. In sharp contrast, all of the aliphatic esters (**3C-3O**) exhibited good to excellent activities in the tubulin and cytotoxicity assays. For example, C4-fluoroacetoxy and C-4 trichloroacetoxy derivatives (**3C** & **3D**) possessed comparable activities to that displayed by paclitaxel. A rather important trend is observed simply by comparing the cytotoxicity value of the following five straight chain (2-6 carbon) carrying analogs: **1** (paclitaxel), **3E**, **3G**, **3L** and **3N**. It is obvious that the 4-butyrate ester (**3G**) is the most potent analog. This trend also indicates that the four-carbon chain is probably the optimal size for the effective receptor-binding. In order to further optimize activity, we decided to synthesize other four-carbon bearing C-4 esters. These included a 4-iso-butyrate derivative (**3H**) and three unsaturated ester analogs (**3I-3K**). Of these four analogs, the 4-cyclopropyl ester (**3K**) is the most potent derivative. In fact, analog **3K** is even more potent than the 4-butyrate ester (**3G**) in both the tubulin polymerization assay as well as binding assay. It is also worthwhile to point out that our result for the 4-iso-butyrate derivative (**3H**) obtained from the tubulin polymerization assay does not match that observed by Georg and her co-workers.[8] These workers reported that analog **3H** was 2.6 times less potent than paclitaxel in a microtubule assembly assay. The C-4 cyclobutyl ester (**3M**) and 4-cyclopentyl derivative (**3O**) were also found to be very potent in the bioassays.
Three C-4 carbonate analogs (**4A-4C**) were found to be universally more potent than paclitaxel in all three bioassys. All three carbonates possessed similar cytotoxicity although the C-4 ethyl carbonate (**4B**) appeared to be the most potent analog.
Of the C-4 carbamates included in Table 1, the C-4-imidazole carrying analog (**5B**) was essentially inactive. On the other hand, the C-4-aziridine derivative (**5C**) exhibited slightly weaker activity in both the tubulin and the cytotoxicity assays, as compared with paclitaxel.[6] The C-4 cyclobutylcarbamate analog (**5A**) showed comparable cytotoxicity to that of paclitaxel, and this analog was also found to be more potent than paclitaxel in the tubulin polymerization assay.
As highlighted in Table 1, several potent analogs of each structure-type were derivatized further on the side chain C-3' and 3'-N positions. Recent reports from Holton,[13] Ojima,[14] Georg[15] and Maring[16] clearly indicate that replacement of 3'C-phenyl with either 3'C-furyl or 3'C-iso-butenyl leads to paclitaxel side chain analogs possessing enhanced *in vitro* activity. Likewise, replacement of 3'-N-benzamide with 3'-N-Boc could also improve drug's potency. A total of five such side chains (**11b-11f**)[13-16] were coupled efficiently onto three C-4

Scheme 1

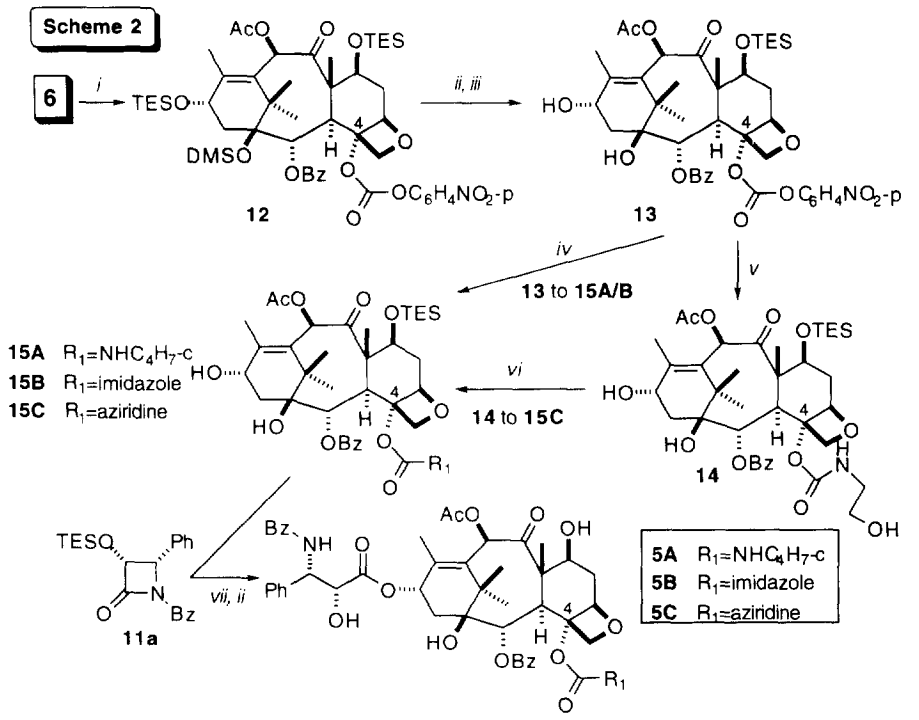


Reagents and Conditions: (i) LHMDS/THF/0 °C, then acyl chlorides or chloroformates, 42-94%; (ii) 48% HF/Pyridine/CH_3CN/0 °C; then TESCl/imidazole/DMF/0 °C, 24-81%; (iii) LHMDS/THF/0 °C, β-lactam (**11a**); then 48% HF/Pyridine/CH_3CN/0 °C, 68-80%.

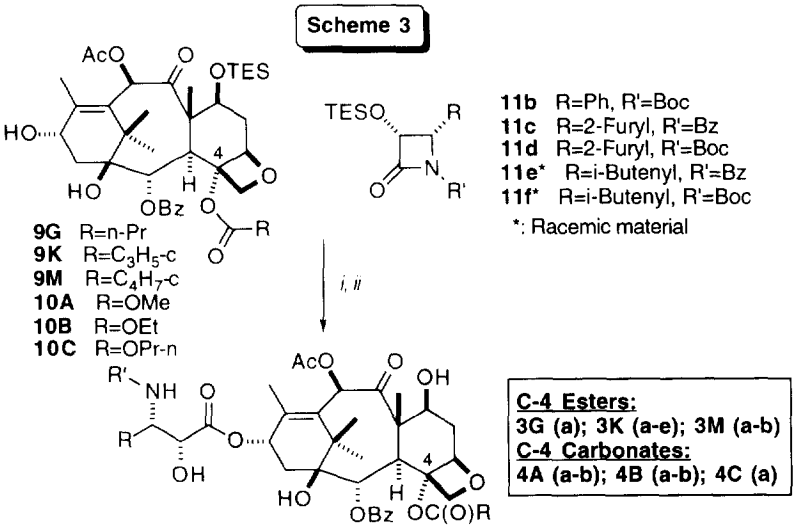
Table 1: Paclitaxel Side Chain Bearing C-4 Analogs 3(A-O), 4(A-C) and 5(A-C):

Analog#	*C-4 Modification*	*Tubulin Poly. ratio**	*IC_{50} (nM)*		*Binding ratio****
			*Ana./Tax.***	*HCT-116*	
1	CH_3	1.0	1.0	2.4-4.0	1.0
2	H	200	>200	>1000	
3A	C_6H_5	>60	103	410	
3B	C6H4F-p	61	198	790	
3C	CH_2F	1.2	1.75	7.0	
3D	CCl_3	0.68	1.1	4.0	0.70
3E	CH_2CH_3	1.5	0.5	2.0	1.30
3F	$CH{=}CH_2$	0.42	1.5	6.0	0.60
3G	**$CH_2CH_2CH_3$**	**0.61**	**0.25**	**1.1**	**0.60**
3H	$CH(CH_3)_2$	0.46	1.25	5.0	n.d.
3I	$C(Me){=}CH_2$	0.71	1.13	4.5	n.d.
3J	transCH=CH(Me)	0.70	0.58	2.3	n.d.
3K	**C_3H_5-c**	**0.24**	**0.25**	**1.0**	**0.48**
3L	$(CH_2)_3CH_3$	1.04	0.5	2.0	0.70
3M	**C_4H_7-c**	**0.44**	**0.38**	**1.5**	**0.90**
3N	$(CH_2)_4CH_3$	3.4	1.5	6.0	3.30
3O	C5H9-c	1.54	0.50	2.0	0.90
4A	**OMe**	**0.41**	**0.50**	**2.0**	**0.70**
4B	**OEt**	**0.64**	**0.25**	**1.0**	**0.56**
4C	**OPr-n**	**0.76**	**0.67**	**2.6**	n.d.
5A	NHC_4H_7-c	0.76	2.3	9.2	
5B	imidazole	263	251	803	
5C	aziridine	2.8	6.5	15.6	

*/**/***: see text for definition.



Reagents and Conditions: (i) LHMDS/THF/0 °C, p-$NO_2C_6H_4OCOCl$, 51%; (ii) pyridine/48%HF/ CH_3CN/5 °C, 96%; (iii) TESCl/imidazole/DMF/0 °C, 90%; (iv) c-$C_4H_7NH_2$/THF/r.t., 86% (**15A**); imidazole/THF/r.t., 76% (**15B**); (v) ethanolamine/THF/r.t., 97%; (vi) DEAD/PPh3/THF,r.t., 49% (**15C**); (vii) LHMDS/THF/-40 °C, (**11a**); then (ii) to afford (**5A-C**).



Reagents and Conditions: (i) (**9G,K,M**) or (**10A,B,C**)/LHMDS/THF/-40 °C, β-lactams (**11b-f**), 64-95%; (ii) pyridine/48%HF/CH_3CN/5 °C, 72-93%.

ester linking baccatin derivatives (**9G**, **9K**, **9M**) as well as three C-4 carbonate linkage containing derivatives (**10A-10C**). These reactions provided thirteen C-4 esters and carbonates for biological evaluation (Scheme 3).

The non-paclitaxel side chain bearing C-4 derivatives were subjected to biological evaluation *in vitro*. Eleven of these analogs were further evaluated in an *in vivo* experiment against i.p. implanted murine M-109 lung carcinoma.[17] The results of these investigations are listed in Table 2 below.

In Vitro Evaluation: *Careful inspection of the results listed in Table 2 reveals again the good correlation between the tubulin polymerization assay and the cytotoxicity assay.* The general conclusion emerging from the *in vitro* data is that side chain modifications do not seem to have significant impact on *in vitro* activities of these C-4 esters and C-4 carbonates listed in Table 2. In fact, none of the 3'-C and/or 3'-N replacements gained clear benefit over the corresponding parent compounds. For example, the following pairs of C-4 esters and carbonates, regardless of their side chain substituents, displayed almost equal potencies in both the tubulin polymerization assay and the cytotoxicity assay against the HCT-116 cell line: 4-butyrate esters (**3G**, **3Ga**); 4-cyclopropyl esters (**3K**, **3Kc**); 4-cyclobutyl esters (**3M**, **3Mb**); 4-methyl carbonates (**4A**, **4Aa**); 4-ethyl carbonates (**4B**, **4Ba**); 4-n-propyl carbonates (**4C**, **4Ca**). Either the assays as conducted are incapable of measuring differences that exist or the potency of the C-4 analogs are not enhanced by side chain changes.

Table 2: Non-Paclitaxel Side Chain Bearing C-4 Analogs 3, 4:

Analog#	*C-4*	*C-3'*	*3'-N*	*Tubulin ratio**	*IC_{50} (nM)*		*i.p.M109*
					*Ana/Tax.***	*HCT-116*	*T/C(mg/Kg/inj.)*
1	*CH3*	*Ph*	*Bz*	*1.0*	*1.0*	*2.4-4.0*	159-228(60)
3G	*n-Pr*	*Ph*	*Bz*	*0.61*	*0.25*	*1.1*	***164(25)***
3Ga	n-Pr	2-Furyl	t-Boc	0.5	0.7	2.8	96(1)
3K	*C3H5-c*	*Ph*	*Bz*	*0.24*	*0.25*	*1.0*	***188(6)***
3Ka	C3H5-c	Ph	t-Boc	0.22	0.20	0.8	115(1.6)
3Kb	C3H5-c	2-Furyl	Bz	0.33	0.22	0.9	**132(3)**
3Kc	C3H5-c	2-Furyl	t-Boc	0.33	0.33	1.3	100(4)
3Kd	C3H5-c	i-Butenyl	t-Boc	0.36	2.85	7.7	n.a.
3Ke	C3H5-c	i-Butenyl	Bz	0.36	0.61	1.7	n.a.
3M	*C4H7-c*	*Ph*	*Bz*	*0.44*	*0.38*	*1.5*	*n.a.*
3Ma	C4H7-c	2-Furyl	Bz	0.29	0.4	1.6	**188(16)**
3Mb	C4H7-c	2-Furyl	t-Boc	0.48	0.48	1.9	n.a.
4A	*OMe*	*Ph*	*Bz*	*0.41*	*0.50*	*2.0*	***161(50)***
4Aa	OMe	2-Furyl	t-Boc	0.66	0.4	1.6	**183(32)**
4Ab	OMe	2-Furyl	Bz	0.33	0.57	2.3	n.a.
4B	*OEt*	*Ph*	*Bz*	*0.64*	*0.25*	*1.0*	***142(6)***
4Ba	OEt	2-Furyl	t-Boc	0.3	0.59	2.95	**144(4)**
4Bb	OEt	2-Furyl	Bz	0.4	0.43	1.5	n.a.
4C	*OPr-n*	*Ph*	*Bz*	*0.76*	*0.67*	*2.6*	*n.a.*
4Ca	OPr-n	2-Furyl	Boc	0.71	0.60	3.0	n.a.

*/**: see text for definition.

In Vivo Evaluation: Of the eleven C-4 esters and carbonates tested against murine M-109 lung carcinoma,[17] eight of them were active (T/C>125%). The best results were obtained with the paclitaxel side chain bearing 4-cyclopropyl ester (**3K**) and two non-paclitaxel side chain containing analogs (**3Ma**, **4Aa**), with T/C (%) values of 188, 188, and 183, respectively. It is also worthwhile to note that some of these C-4 analogs were 10-15 times more potent than paclitaxel. These include two C-4 cyclopropyl esters (**3K**, **3Kb**) and two C-4 methyl carbonate derivatives (**4B**, **4Ba**). However, the limitation is that none of these C-4 analogs was found to be more efficacious than paclitaxel [T/C=159-228 %]. Moreover, attempts to improve the *in vivo* efficacy by means of further side chain modifications were not successful. For instance, 3'-furyl/3'-N-Boc carrying analogs (**3Ga** and **3Kc**) possessed reduced activities as compared with their parent analogs (**3G** and **3K**). In fact, none of the C-4 cyclopropyl ester analogs prepared in Scheme 3, **3Ka-3Ke**, showed any improvement over parent compound (**3K**) *in vivo*. On the other hand, C-4 ethyl carbonate **4Aa** exhibited slightly better activity than the paclitaxel side chain bearing parent analog **4A**. No side chain effect was seen with C-4 ethyl carbonate analogs. In view of these discrepancies, it is thus fair to say that side chain modification provided at best only minimal improvements in *in vivo* efficacy.

In summary, a large number of C-4 esters, carbonates and carbamates synthesized in our laboratory[18] were highly cytotoxic to cancer cells incubated. Some of these analogs (e.g. 4-butyrate ester **3G**, 4-cyclopropyl ester **3K** and 4-cyclobutyl ester **3Ma**, 4-methyl carbonate derivative **4Aa**) also possessed activities against murine i.p. M-109 lung carcinoma *in vivo*. Several of these analogs achieved their optimal effect at doses almost ten fold lower than paclitaxel and thus more potent. These promising results are in good agreement with our previous assumption that the C-4 substituent is involved in the imtimate tubulin binding, therefore, modification of this group holds great promise for discovery of new analogs with altered properties. It is also important to recognize that *in vitro* potency , as reflected by IC_{50} value versus HCT-116 cell lines, does not always correlate well with *in vivo* efficacy as indicated by T/C value (c.f. data for analogs **3Ga**, **3Ka**, **3Kc**). Therefore, the *in vivo* data are necessary for the evaluation of paclitaxel analogs. This conclusion parallels the recent results of Nicolaou's group with C-2 taxoids[19] as well as our own finding with C-7 paclitaxel derivatives.[20]

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