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TAXOL® STRUCTURE-ACTIVITY RELATIONSHIPS: SYNTHESIS AND BIOLOGICAL EVALUATION OF TAXOL ANALOGS MODIFIED AT C-7

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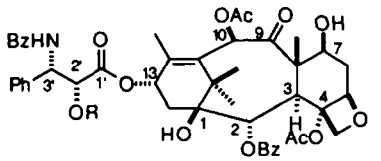
Abstract: A series of taxol derivatives, modified at C-7 , is described. This includes sulfonate, silylether, ester, carbonate, carbamate, fluoro, dehydro and deoxy derivatives. Biological evaluation shows that these modifications do not usually significantly compromise activity. However, none of the C-7 analogs prepared thus far have been shown to be better than taxol in both in vitro and in vivo assays.

INTRODUCTION

The structurally unique diterpenoid paclitaxel (taxol®**1**) was isolated from the western yew *Taxus brevifolia* by Wani and Wall in 1971.[1] This compound has potent anticancer and antileukemic properties, and shows good activity against ovarian, lung and breast cancers.[2] Another interesting feature of paclitaxel is that it acts in an unusual way, i.e. it promotes tubulin polymerization even in the absence of cofactors (GTP), thus disrupting the tubulin-microtubule equilibrium and consequently inhibiting mitosis.[3] Based on these exciting clinical and pharmacological results, paclitaxel is clearly one of the most important anti-cancer drugs of this decade. Structure-activity relationship studies have been reviewed recently.[4a-c]



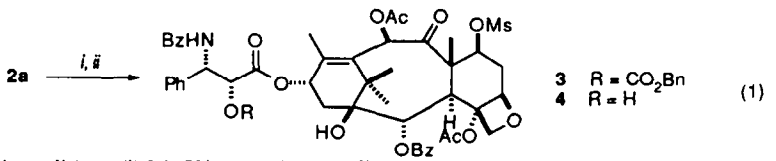
1 R = H ***Taxol***
2a R = CO_2Bn
2b R = TES
2c R=$COOCH_2CH=CH_2$

It has been shown earlier that modifications at C-7 have minimally affected the biological activities, i.e. tubulin binding and cytotoxicity.[4] Acetylation at C-7 does not significantly alter the cytotoxicity, while epimerization at C-7 only slightly reduces the *in vitro* activity.[4a] We have recently shown that the C-2 benzoate residue is very important for biological activity,[5] but that the acetoxy group at C-10 is not crucial.[6] As an extention of our SAR efforts, we decided to prepare more derivatives modified at C-7 in order to confirm that this position is not involved in interactions at the binding site. While one would not expect to produce more strongly binding analogs by modifiying an unessential moiety, modifications at C-7 may affect other properties of the drug (such as membrane permeability, metabolic stability, binding kinetics) and therefore influence the overall performance of the drug favorably.

CHEMISTRY

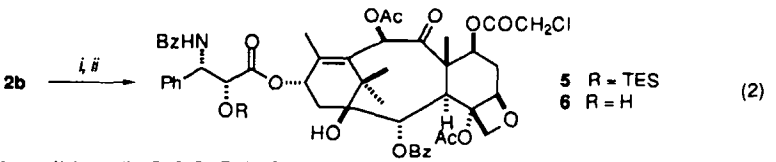
Mesylate **3** was prepared by treatment of **2a** with a large excess (30 eq) of methanesulfonyl chloride in pyridine at room temperature (Eq.1). Standard conditions (use of 1 eq. MsCl) failed to produce the desired mesylate presumably due to steric hindrance at C-7. Catalytic hydrogenation of **3** yielded the desired compound **4** in good yield.

Kingston has described the synthesis of 7-acetyl taxol.[7] We were interested in preparing esters that may lead to covalent binding to tubulin, i.e. affinity probes.[8] Ester **6** was prepared from **5** by standard acylation followed by desilylation (Eq. 2).



(1)

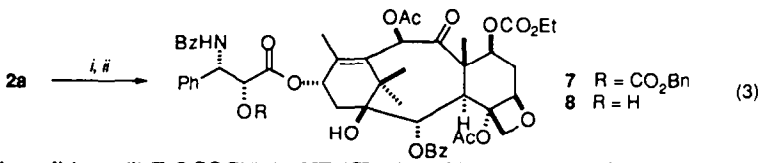
Reagents and conditions:(i) MsCl/pyr/DMAP (74%); (ii) H_2/Pd/C/EtOAc (85%)



(2)

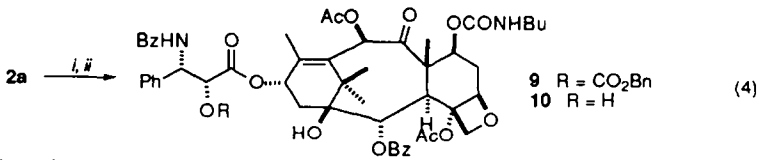
Reagents and conditions:(i) $ClCH_2C(O)Cl$/pyr/CH_2Cl_2 (100%); (ii) TBAF/THF (79%)

Carbonate derivatives at C-7 of taxol have not yet been reported. The synthesis of the ethyl carbonate **8** was accomplished by hydrogenation of carbonate **7**, which was prepared in turn from **2a** by treatment with ethyl chloroformate and diisopropylethylamine in dichloromethane (Eq. 3).



(3)

Reagents and conditions: (i) EtOCOCl/i-Pr_2NEt/CH_2Cl_2 (64%); (ii)H_2/Pd/C/ EtOAc (70%)



(4)

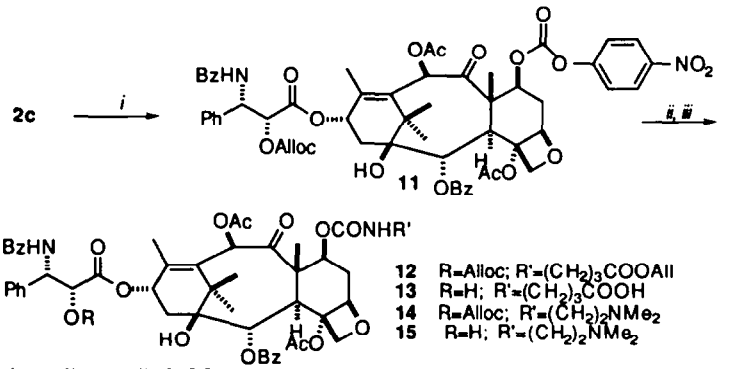
Reagents and conditions: (i) $COCl_2$/pyr/n-$BuNH_2$ (72%); (ii) H_2/Pd/C/EtOAc (90%).

The synthesis of C-7 carbamates turned out to be a rather challenging undertaking since standard methods (treatment of **2a** with isocyanates) failed. After some experimentation, we found that treatment of **2a** with phosgene afforded the chloroformate, which was then converted to **9** by addition of butylamine (Eq. 4). Alternatively, the p-nitrophenyl carbonate at C-7 (**11**) could be easily prepared, and this intermediate was used successfully for the preparation of more complex carbamates like **13** and **15**. (Eq. 5)

Replacement of the C-7 hydroxyl group with fluorine was described in a preliminary communication.[9]

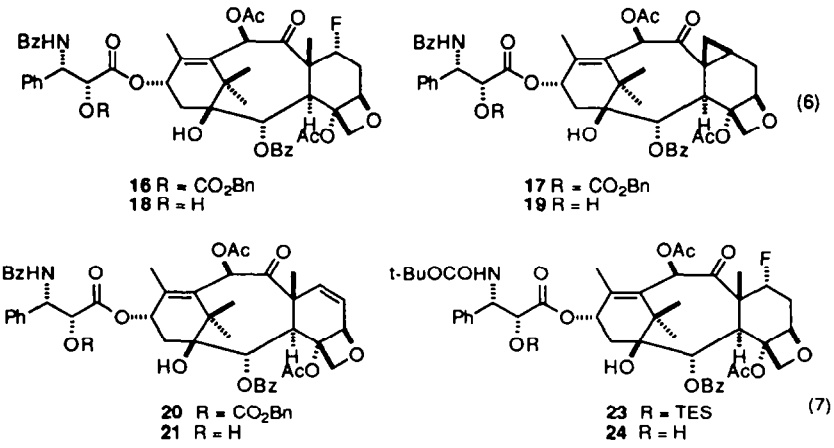
Treatment of a dichloromethane solution of **2a** with DAST yielded 7-(α)-fluoro derivative **16** (55%) together with 7,19-cyclopropane **17** (32%). Standard hydrogenation of these compounds with palladium on charcoal yielded **18** (88%) and **19** (90%), respectively (Figure 6).

Interestingly, when the fluorination reaction was performed in ethereal solvents, such as THF and diethylether, the dehydration product **20** (12%) was isolated in addition to the fluoro derivative **16** (51%) and cyclopropane **17** (2-3%). Standard hydrogenation of **20** with palladium on charcoal yielded the novel 6,7-dehydropaclitaxel (**21**) (Figure 7).



(5)

Reagents and conditions:(i) $ClCO_2C_6H_4NO_2$-p/pyr/CH_3CN (78%);(ii) **12**: H_2N-$(CH_2)_3$-COOAll, THF (93%); **14**: H_2N-$(CH_2)_2$-NMe_2, THF (91%). (iii) Pd_2dba_3, CH_2Cl_2, PPh_3, triethanolamine; 68% for **13**; 65% for **15**.



(6)

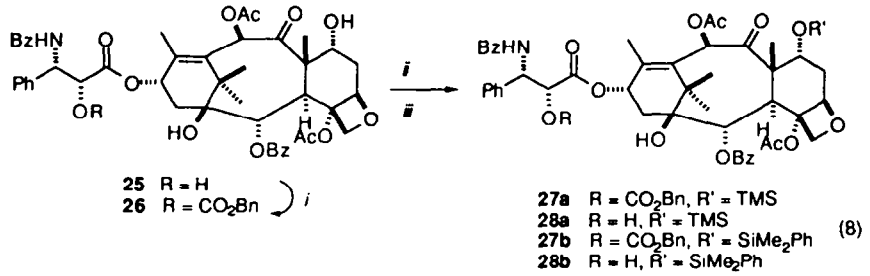
(7)

In order to ascertain the effect of a different side chain in conjunction with modified cores, 7-(α)-fluorodocetaxel **24** was prepared by standard coupling (84%) of 7-fluorobaccatin III, **22**, with the appropriate β-lactam,[10] followed by desilylation (TBAF/THF, 76%). C7-fluorobaccatin III (**22**) was prepared from 7-fluoropaclitaxel (**18**) via borohydride-mediated side chain cleavage (93%).[7]

7-Epi paclitaxel is known to be very inert to chemical reactions,[7] in part because of its hydrogen bonding to the C-4 acetate.[11] Indeed, no functional modifications of this group have been reported to date. Assuming that polar solvents should be able to break such intramolecular hydrogen bond, we treated compound **26** with chlorotrimethylsilane or chlorodimethylphenylsilane and imidazole in DMF (Eq. 8). 7-Epi silyl ethers **27a** and **27b** were formed respectively. Deprotection (H_2/Pd/C) afforded **28a,b** for biological testing. Similarly, C-7 (β)-triethylsilyl paclitaxel (**30**) was prepared for biological evaluation. The synthesis of **30** is outlined below (Eq. 9).

A direct way to probe the role of the C-7 hydroxyl group in interacting with the tubulin binding site is to replace that functionality with a hydrogen atom. We and Kingston have recently reported on the

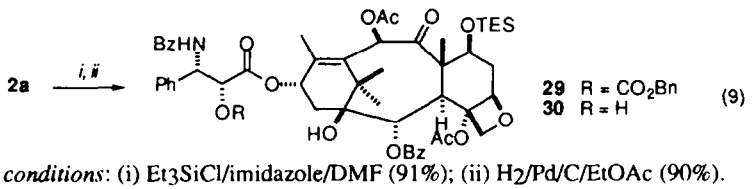
chemistry needed for such transformation.[12] The docetaxel analog of 7-deoxypaclitaxel was also prepared by acylation of the baccatin derivative **31** with the appropriate side chain, as shown Eq. 10.



25 R = H
26 R = CO_2Bn

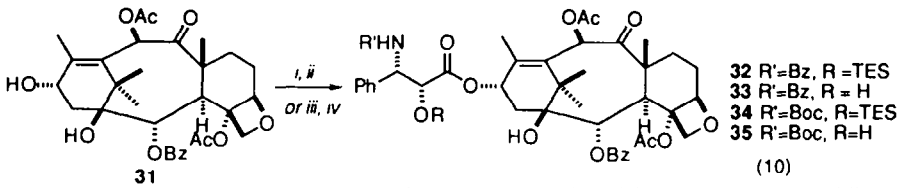
27a R = CO_2Bn, R' = TMS
28a R = H, R' = TMS
27b R = CO_2Bn, R' = $SiMe_2Ph$
28b R = H, R' = $SiMe_2Ph$ (8)

Reagents and conditions: (i) CbzCl/i-Pr_2NEt/CH_2Cl_2 (88%); (ii)TMSCl/imid./DMF **27a** (79%); or $Me_2PhSiCl$/imid./DMF **27b** (76%); (iii)H_2/Pd/C/EtOAc **28a** (92%); **28b** (89%).



29 R = CO_2Bn
30 R = H (9)

Reagents and conditions: (i) Et_3SiCl/imidazole/DMF (91%); (ii) H_2/Pd/C/EtOAc (90%).



31

32 R'=Bz, R =TES
33 R'=Bz, R = H
34 R'=Boc, R=TES
35 R'=Boc, R=H (10)

Reagents and conditions: (i) n-BuLi/THF/(3R,4S)-1-benzoyl-3-triethylsilyloxy-4-phenylazetidinone (87%); (ii) TBAF/THF for **33** (84%); (iii) n-BuLi/THF/(3R,4S)-1-t-butoxycarbonyl-3-triethylsilyloxy-4-phenylazetidinone (95%); (iv) TBAF/THF for **35** (88%).

BIOLOGY

A total of sixteen C7 paclitaxel analogs were evaluated in a tubulin polymerization assay[13] and an *in vitro* cytotoxicity assay against a human colon cancer cell line.[14] Five of those potent analogs were evaluated further in an *in vivo* assay against the murine M109 lung carcinoma.[15]

Ratio of initial tubulin polymerization rate measures the potency of analog relative to taxol and ratios >1 signifies analog being more potent.[13] The *in vitro* IC_{50} measures the drug concentration required for the inhibition of 50% cell proliferation after a 72 hours incubation.[14] The ip/ip tumor model used here serves as an initial screen for *in vivo* activity to determine if further evaluations in other tumor models are justified.[15] (see Table 1)

All of the potent analogs in the cytotoxicity assay, ranging from more potent to three-fold less potent than taxol, are listed in Table 1. In general, 7-mesylate, 7-ethylcarbonate, 7-chloromethylacetate, 6,7-dehydro, 7-fluorinated, 7-deoxygenated, 7-epimer as well as 7,19-cyclopropane taxol analogs were shown to be quite potent in our cytotoxicity assay. It is worthy of knowing that the most potent analog shown here, the taxotere side chain bearing C-7 fluorotaxol **35** was three times more potent than paclitaxel. Three

analogs were found to be able to polymerize tubulin better than taxol and they are 7-mesylate (**4**), 7-epi-fluoropaclitaxel (**18**) and 6,7-dehydropaclitaxel (**21**). It is interesting to note that another three analogs with poor activity in the tubulin assay, 7,19-cyclopropanepaclitaxel (**19**), 7-epi-paclitaxel (**25**) and 7-deoxypaclitaxel (**33**), completely retained their activities in the cytotoxicity assay. Thus, careful examination of the biological data shown in Table 1 reveals that the replacement of C-7 hydroxyl group with small size substituents, such as, for example, fluorine and hydrogen, does not significantly affect the *in vitro* potency.[16]

***Table 1:** List of C7 analogs with in vitro cytotoxicity comparable to taxol*

Compound	Description	Tubulin Poly. init. rate ratio	IC_{50} (nM) HCT 116	ip/ip in vivo Max. T/C(mg/kg/inj)
Paclitaxel	--	1.00	4.0	183-276(50-75)
Docetaxel	--	2.00	3.0	Scheduled
4	7-mesylate paclitaxel	**1.10**	n.d.	n.d.
6	7-chloromethylester paclitaxel	0.81	7.0	n.d.
8	7-ethylcarbonate paclitaxel	0.72	6.0	**289(40)**
18	7-epi-fluoro paclitaxel	**1.45**	11	185(132)
19	7,19-cyclopropane paclitaxel	0.56	8.0	156(80)
21	6,7-dehydro paclitaxel	**1.31**	5.0	161(60)
24	7-epi-fluoro-3'-N-Boc paclitaxel	0.80	4.7	147(40)
25	7-epi-paclitaxel	0.45	**2.0**	126(30), 154(32)
33	7-deoxy paclitaxel	0.23	4.0	157(50)
35	7-deoxy-3'-N-Boc paclitaxel	0.80	**1.6**	156(64)

Eight of those potent analogs whose *in vitro* data are described in Table 1 were also evaluated *i.p. in vivo* versus the *i.p.*-implanted M109 tumor. All eight analogs were active (T/C≥125%). In the unstaged M109 tumor model, with treatment indicated on Day 1 post-implant, 7-ethylcarbonate paclitaxel (**8**) was nearly as active as concomitantly evaluated paclitaxel (T/C values of 289% and 336%, respectively) and their potencies, based on the optimal doses in that experiment, were also similar. In the eight subsequent experiments performed and reprented in Table 1, treatments were administered on Day 5 and 8 post-implant. The maximum T/C values obtained with paclitaxel ranged from 183%-276%, at optimal doses of 50-75 mg/kg/inj. In comparison, the seven analogs produced maximum T/C values of between 126-185%, at optimal doses of between 30-132 mg/kg/inj. The most potent but least active analog was 7-epi paclitaxel (**25**), producing T/C values of 126 and 154% in two separate experiments, at optimal doses of 30-32 mg/kg/inj, respectively. Analog **18**, 7-epi-fluoropaclitaxel, was the least potent compound, with an optimal dose of 132 mg/kg/inj, but it was reasonably active (T/C of 185%). *In vitro* potency, as reflected by IC_{50} values versus HCT-116 cells, correlated somewhat with *in vivo* potency as reflected by optimal dose, but neither potency *in vitro* nor *in vivo* predicted well for activity *in vivo* (c.f. data for analogs **18** and **25**).

***Table 2:** List of C7 analogs with poor in vitro cytotoxicity*

Compound	Description	Tubulin Polymer. init. rate ratio	IC_{50} (nM) HCT 116
Paclitaxel	--	1.00	4.0
10	7-butylcarbamate	0.96	46
13	--	0.71	>3900
15	--	0.40	>100
28a	7-α-trimethylsilyether	0	>60
28b	7-α-dimethylphenylsilyether	0	>78
30	7-β-triethylsilyether	0.53	>78

As can be seen in Table 2, three C7 carbamates analogs, **10**, **13** and **15**, possessed marked reduced cytotoxicity in comparison to paclitaxel. Interestingly, all these carbamates still retained their activity in the tubulin polymerization assay. Similar results were also obtained by Stella with the C-7 aminoacid ester analogs bearing dimethylamino or carboxylic acid terminus.[4d] These observations seems to suggest that the lack of potency in the cytotoxicity assay may be due to the incapablity of these analogs to get

into the cells. Two C-7-epi-silyether analogs, **28a** and **28b**, were found to be inactive in both assays. This clearly indicates that the presence of bulky substitutes in the α-face of the molecule will erode the receptor binding. Not surprisingly, the bulky 7β-triethylsilylether analog, **30**, did not possess good cytotoxicity, presumably due to the relative large size of the triethylsilyl (TES) group.

In summary, our data shows that the modifications at C-7 position can significantly alter either the *in vitro* or the *in vivo* activity of paclitaxel. None of the C-7 analogs described here exhibited a clear advantage over paclitaxel in all of the three assays tested. As can be seen in Table 1, the results from *in vitro* assay does not correlate with the *in vivo* experiment, therefore, the *in vivo* data is absolutely needed for the evaluation of paclitaxel analogs.

REFERENCES AND NOTES

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¶ Taxol® is the registered trademark of Bristol-Myers Squibb company.

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