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SYNTHESIS AND BIOLOGICAL ACTIVITY OF 14-HYDROXYDOCETAXEL

Iwao Ojima†*, Ivana Fenoglio†, Young Hoon Park†, Paula Pera‡, Ralph J. Bernacki‡
†*Department of Chemistry, State University of New York at Stony Brook, Stony Brook, New York 11794-3400.*
‡*Department of Experimental Therapeutics, Grace Cancer Drug Center, Roswell Park Cancer Institute, Elm and Carlton Streets, Buffalo, New York 14263*

Abstract. New taxoids, 14β-hydroxydocetaxel and its 1,14-acetonide, are synthesized from 14β-hydroxy-10-deacetylbaccatin III in good yields. The cytotoxicity of the new taxoids are evaluated against different human tumor cell lines and their ability to inhibit the microtubules disassembly examined. 14β-hydroxydocetaxel shows very strong cytotoxicity, especially against A549 human non-small cell lung cancer cell line (IC_{50} = 0.8 nM).

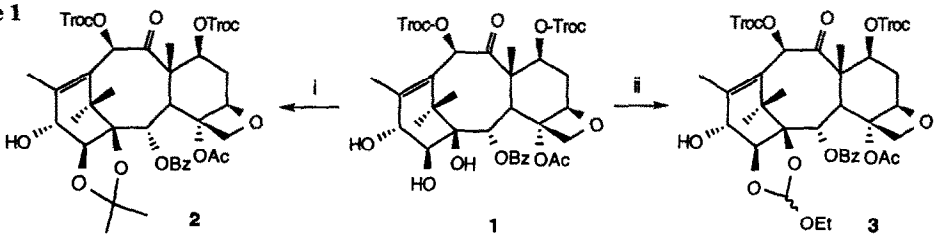
Taxol® (paclitaxel) is currently considered the most exciting lead in cancer chemotherapy.[1-3] Taxotere® (docetaxel), a semisynthetic analog, is also very promising.[4] Paclitaxel and docetaxel possess strong antitumor activity against different cancers which have not been effectively treated by existing antitumor drugs.[5,6] Paclitaxel has been approved by FDA in late 1992 for the treatment of advanced ovarian cancer, and is currently in phase II and III clinical trials for breast cancer, lung and other cancers.[2] Docetaxel is currently in phase II and III clinical trials for breast and lung cancers in the United States, Europe, and Japan and expected to be on the market very soon.[6] A recent report on clinical trials of paclitaxel and docetaxel, however, has disclosed that these highly effective drugs have a number of undesired side effects as well as multi-drug resistance (MDR).[1,5,7] Therefore, it is very important to develop new anticancer drugs which have less undesirable side effects, better pharmacological properties, and/or activity spectra against various tumor types different from those of these two drugs.

14β-Hydroxy-10-deacetylbaccatin III (14-OH-DAB), recently isolated from the needles of *Taxus wallichiana Zucc.*[8] possesses an extra hydroxyl group at the C-14 position, which makes 14-OH-DAB much more water soluble than the usual 10-deacetylbaccatin III (DAB) which is the key intermediate for paclitaxel and docetaxel. Therefore, the new antitumor taxanes derived from 14-OH-DAB can be expected to have improved water solubility, bioavailability, and hydrophobicity-related drug resistance.[7] These improved pharmacological properties may well be related to the modification of undesirable toxicity and activity spectra against different cancer types. In fact, recent reports on the related "hydroxy-taxoids", 9-dihydrodocetaxel[9] and 19-hydroxydocetaxel[10], show quite promising results. We would like to communicate here the synthesis of a highly potent new taxoid, 14β-hydroxydocetaxel (**SB-T-1001**), and a related analog, 14β-hydroxydocetaxel-1,14-acetonide (**SB-T-1071**).[11]

Syntheses of 14β-hydroxy-docetaxel-1,14-acetonide (SB-T-1071) and 14β-hydroxydocetaxel (SB-T-1001). We have found that the reactivities of the four secondary hydroxyl groups of 14β-OH-DAB toward acylation decreases in the order C-7 > C-10 > C-14 > C-13, which appears to be in good agreement with the relative steric congestion at these positions. Accordingly, the coupling of the baccatin in 13-position with an

isoserine side chain precursor requires an appropriate protection of hydroxyl groups at the 7, 10 and 14 positions. The selective protection of the 7- and 10-hydroxyl groups was easily carried out using 2,2,2-trichloroethyl chlorocarbonate (troc-Cl) in the presence of pyridine to give 7,10-ditroc-14β-hydroxy-10-deacetylbaccatin III (**1**) in high yield.[12] However, the protection of the 14-hydroxyl group was not straight forward, e.g., (i) an attempted protection with troc-Cl/pyridine resulted in the formation of 1,14-carbonate,[12] (ii) the selective protection with triethylsilyl (TES) was successful, but the attempted coupling with a C-13 side chain precursor, a β-lactam **4** (*vide infra*), did not work because of steric hindrance caused by the introduction of TESO group adjacent to the 13-hydroxyl group. We successfully introduced an acetonide as a potential protecting groups for the 1- and 14-hydroxyl groups which constitute cis-diol, giving 7,10-ditroc-14β-hydroxy-10-deacetylbaccatin III-1,14-acetonide (**2**) in 89% yield (Scheme 1), but the attempted deprotection of acetonide under acidic conditions led to the ring opening of the D ring. Thus, acetonide turned out to be inappropriate as the protecting group for our purpose. Finally, we introduced an orthoformate to the 1- and 14-hydroxyl groups using triethyl orthoformate in the presence of *p*-toluenesulfonic anhydride, giving 7,10-ditroc-14β-hydroxy-1,14-ethoxymethylene-10-deacetylbaccatin III (**3**) in 93% yield (Scheme 1). This protecting group was found to be quite appropriate for the coupling with the C-13 side chain precursor **4** (*vide infra*) and subsequent deprotections to give the desired 14β-hydroxydocetaxel (**SB-T-1001**).

Scheme 1

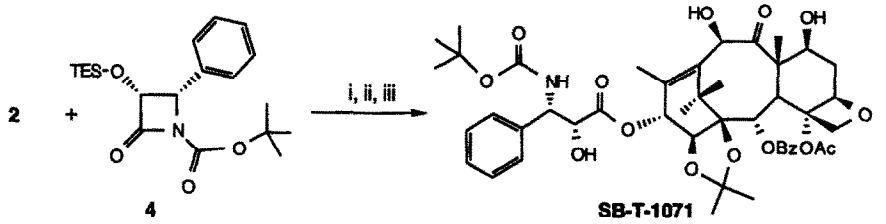


(i) $Me_2C(OMe)_2$, TsOH, room temp., 30 h; (ii) $HC(OEt)_3$, Ts_2O, CH_2Cl_2, room temp., 30 min.

The protected 14-OH-DAB **2** was coupled with enantiopure (3*R*,4*S*)-1-t-BOC-3-TESO-4-phenylazetidin-2-one (**4**) using our protocol,[13] i.e., NaHMDS in THF at -40 °C for 45 min, to give the corresponding coupling product in 82% yield. Since the 1,14-acetonide moiety of this compound cannot be removed without affecting the D-ring, the TES at 2' position and the troc groups at the 7 and 10 positions were removed sequentially by treating with 0.5*N* hydrochloric acid – THF (1:4) and then with zinc in 0.5*N* HCl – THF (1:4), respectively to give 14β-hydroxydocetaxel-1,14-acetonide (**SB-T-1071**)[14] in 72% overall yield (Scheme 2).

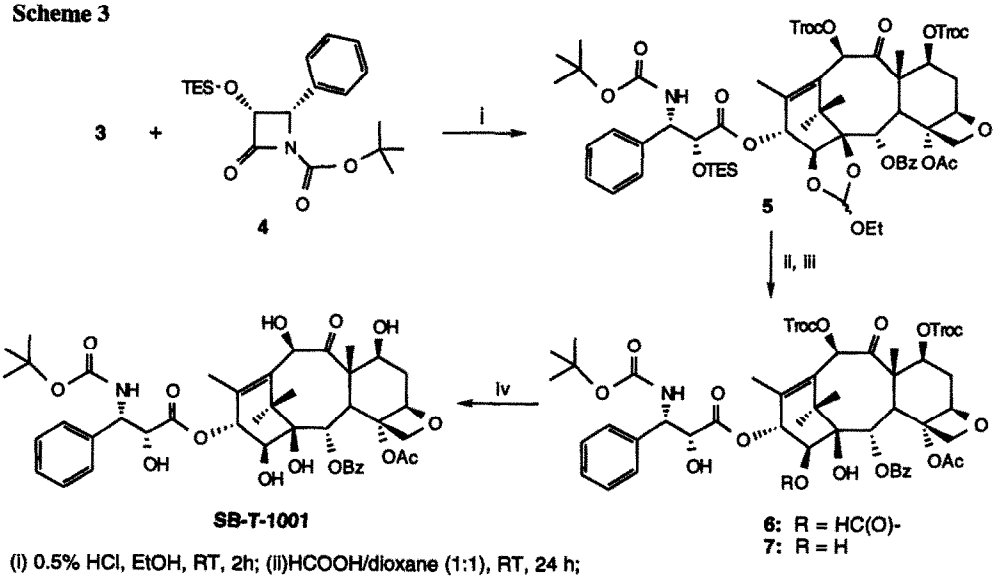
In the same manner, the protected 14-OH-DAB **3** was coupled with **4**, giving **5** in 78% yield. Then, **5** was first treated with 0.5% HCl in EtOH to remove the TES group at the 2' position (96%), followed by reacting with formic acid in dioxane at room temperature for 24 h, yielding 14-formyl-7,10-ditroc-14-hydroxydocetaxel (**6**), which was treated with 1% aqueous $NaHCO_3$ in THF–MeOH (1:7:3) at room temperature for 5 h to give 7,10-ditroc-14-hydroxydocetaxel (**6**, 73% for two steps). The troc groups of **6** at the 7 and 10 positions were deprotected by using zinc in 0.5*N* HCl – THF (1:2) at 0 °C for 30 min to give 14β-hydroxydocetaxel (**SB-T-1001**)[15] in 73% yield (Scheme 3).

Scheme 2



(i) NaHMDS, THF, -40°C, 30 min; (ii) 0.5% HCl in EtOH, RT, 2 h; (iii) Zn, 0.5 N HCl/THF, 0°C, 20 min.

Scheme 3



(i) 0.5% HCl, EtOH, RT, 2h; (ii)HCOOH/dioxane (1:1), RT, 24 h;
(iii) 1% $NaHCO_3$/ THF/MeOH (1:7:3), RT, 5 h; (iv) Zn, 0.5 N HCl/THF (2:1), 0 °C, 30 min.

Cytotoxicity and microtubules disassembly inhibitory activity of new taxoids. Cytotoxicity of 14-OH-docetaxel (**SB-T-1001**) and 14-OH-docetaxel-1,14-acetonide (**SB-T-1071**) were evaluated *in vitro* against different human tumor cell lines A121, A549, HT-29, and MCF7. Microtubules disassembly inhibitory activity of these new taxoids were also examined. Results are summarized in Table 1.

As Table 1 shows, 14-OH-docetaxel (**SB-T-1001**) possesses strong cytotoxicity in between docetaxel and paclitaxel except for the activity against A549 human non-small cell lung cancer cell line (IC_{50} = 0.8 nM) which is slightly better than that of docetaxel. It should be noted that the introduction of 14β-hydroxyl group to docetaxel influences tumor specificity, i.e., 14-OH-docetaxel (**SB-T-1001**) has a higher tumor specificity than docetaxel and paclitaxel. *Although it is preliminary, this result is very encouraging from a view point that structural modifications of docetaxel and paclitaxel can alter the activity spectrum against different tumor types, which may complement the existing anticancer drugs.* 14-OH-Docetaxel-1,14-acetonide (**SB-T-1071**), however, showed one order of magnitude weaker activity in both cytotoxicity and microtubules disassembly inhibitory activity. This can be ascribed to conformational change caused by the bulky acetonide moiety.

Table 1. Cytotoxicity and microtubules disassembly inhibitory activity of **SB-T-1001** and **SB-T-1071**

Cell Line / Taxoid	A121[a] (human ovarian)	A549[a] (human NSCLC)	HT-29[a] (human colon)	MCF7[a] (human breast)	Microtubules disassembly inhibition[b]
Paclitaxel	6.1	3.6	3.2	1.7	1.0T
Docetaxel	1.2	1.0	1.2	1.0	0.7T
SB-T-1001	3.3	0.8	2.1	1.9	0.8T
SB-T-1071	46	18	21	34	3T

[a] The concentration of compound which inhibit 50% (IC_{50}, nM) of the growth of human tumor cell line, A121 (ovarian carcinoma), A549 (non-small cell lung carcinoma), HT-29 (colon carcinoma), and MCF7 (mammary carcinoma) after 72 h drug exposure according to the method developed by Skehan et al.[17] The data represent the mean values of at least three separate experiments. b IC_{50}/IC_{50} (paclitaxel): T = IC_{50} of paclitaxel

In fact, molecular modeling study on the conformations of docetaxel[16] and these two new taxoids based on the SYBYL 6.0 program and NOESY analyses (DMSO-D_2O) clearly indicates the distortion in the "hydrophobic cluster"[16] of 14-OH-docetaxel-1,14-acetonide (**SB-T-1071**) (Figure 1). It should be noted that the introduction of 14β-hydroxyl group to docetaxel has only a little effect on the overall conformation of the molecule although there was a possibility that this hydroxyl group might have significantly changed the side chain conformation through hydrogen bonding. It is also worth mentioning that the conformation of 14-OH-docetaxel (**SB-T-1001**) shows excellent overlap with that of paclitaxel rather than docetaxel as shown in Figure 1.

Further studies on the structure-activity relationships of new taxoids derived from 14-OH-DAB and the conformational analyses of these taxoids based on 1D/2D NMRs and molecular modeling are actively underway in these laboratories.

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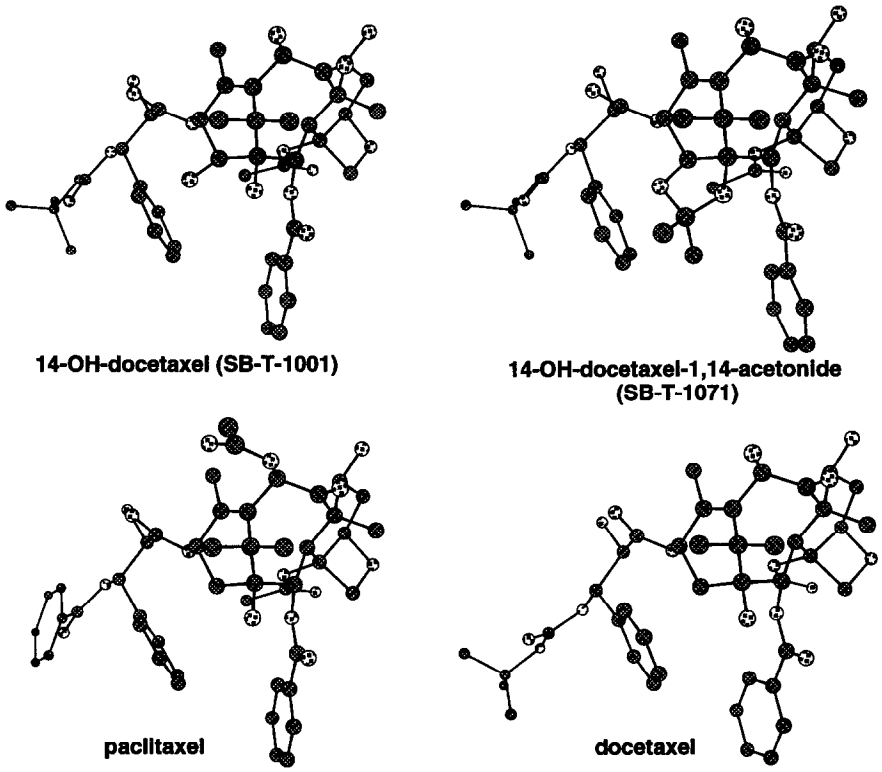

Figure 1. Chem 3D representations of **SB-T-1001**, **SB-T-1071**, paclitaxel and docetaxel

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14. White solid; mp 193-196°C; $[\alpha]^{20}_D$ -57° (c 1, CH_2Cl_2); ^1H NMR ($CDCl_3$) δ 1.01 (s, 3H), 1.22 (s, 6H), 1.38 (s, 3H) (H16, H17 Me acetonide), 1.49 (s, 9H, Boc), 1.71 (s, 3H, H19), 1.75 (s, 3H, H18), 1.83 (m, 1H, H6a), 2.38 (s, 3H, OAc), 2.62 (m, 1H, H6b), 3.74 (bs, 1H, OH), 3.84(d, J = 6.0, 1H, H3), 4.14 (d, J = 8.4, 1H, H20a), 4.1 (m, 1H, H7), 4.19 (bs, 1H, OH), 4.29 (d, J = 4.5, 1H, H14), 4.46 (d, J = 8.4, 1H, H20b), 4.70 (bs, 1H, H2'), 4.97 (bd, J = 8.7, 1H, H5), 5.24 (s, 1H, H10), 5.3 (m, 1H, H3'), 5.79 (bd, J = 9.4, 1H, NH), 6.04 (d, J = 6.0, 1H, H2), 6.26 (bs, 1H, H13), 7.3-7.5 (m, 7H, *m*-Bz, *o,m,p*-Ph), 7.61 (t, J = 7.3 1H, *p*-Bz), 8.09 (d, J = 7.5, 2H, *o*- Bz); ^{13}C ($CDCl_3$) δ 9.34 (C19), 14.68 (C18), 21.07 (C17), 22.60 (OAc), 26.87, 27.57, 27.92 (C16, Me acetonide), 28.31 (Me Boc), 37.54 (C6), 43.16 (C15), 47.35 (C3), 56.06 (C3'), 58.44 (C8), 69.69 (C2), 772.11 (C7), 74.24 (C2'), 74.80 (C10), 77.51 (C20), 78.03 (C13), 80.16 (C14+C4), 81.68 (C, Boc), 84.01 (C5), 88.25 (C1), 111.00 (CH acetonide), 127.07, 127.88, 128.56, 133.64, (*m,p*-Bz, *o,m,p*-Ph), 129.30 (C1 Bz), 130.40 (*o*-Bz), 135,41, 137.41, 138.17, (C12, 11, C1Ph), 155.17 (C=O Boc), 165.16 (C=O Bz), 170.87 (C=O Ac), 171.31 (C 1'), 210.68 (C9). Anal. calcd. for $C_{46}H_{57}O_{15}N$: C, 63.95; H, 6.65; N, 1.62. Found: C, 63.91; H, 6.41; N, 1.50.
15. White solid; mp 193-196°C; $[\alpha]^{22}_D$ -19° (c 1, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.17 (s, 3H, H16), 1.24 (s, 3H, H17), 1.34 (s, 9H, Boc), 1.81 (s+m, 4H, H19+H6a), 1.92 (s, 3H, H18), 2.38 (s, 3H, Ac), 2.59 (m, 1H, H6b), 3.35 (bd, J = 4.2 Hz, 1H, OH), 3.88 (d, J =7.2 Hz, 1H, H3), 4.19 (dd, J =4.2, 7.8 Hz, 1H, H14), 4.21 (dd, J = 6.6, 10.8 Hz, 1H, H7), 4.27 (bs, 1H, OH), 4.28 (d, J = 8.4 Hz, 1H, H20a), 4.37 (d, J = 8.4 Hz, 1H, H20b), 4.48 (bs, 1H, OH), 4.80 (bd, J =3.9 Hz, 1H, H2'), 4.94 (bd, J = 9.0 Hz, 1H, H5), 5.18 (s, 1H, H10), 5.48 (bd, J =10.2 Hz, 1H, H3'), 5.56 (bd, J =10.2 Hz, 1H, NH), 5.62 (d, J = 4.2 Hz, 1H, OH), 5.87 (d, J =7.5 Hz, 1H, H2), 6.21 (bd, J = 7.8 Hz, 1H, H13), 7.3-7.5 (m, 7H, *m*-Bz, *o,m,p*-Ph), 7.58 (t, J = 7.1 Hz, 1H, *p*-Bz), 8.12 (d, J = 7.2 Hz, 2H, *o*-Bz); ^{13}C ($CDCl_3$) δ 10.01 (C19), 14.35 (C18), 22.49 (C17), 22.66 (Ac), 26.33 (C16), 28.24 (Boc) 36.81 (C6), 43.10 (C15), 45.61 (C3), 55.41 (C3'), 57.69 (C8), 69.35 (C14), 71.88 (C7), 72.35 (C2'), 74.31 (C10), 74.51 (C2), 76.24 (C4), 76.53 (C20), 80.53 (C13), 81.22 (Boc), 81.81 (C1),84.04 (5), 126.70, 128.26, 128.59, 129.05, 133.23, (*m, p*-Bz, *o,m,p*-Ph) 129.74 (C1 Bz), 134.56 , 138.07, 133.23 (C11, C12, C1Ph), 157.06 (C=O Boc), 166.07 (C=O Bz), 169.83 (C=O Ac), 173.49 (C1'), 211.13 (C9); Anal. calcd. for $C_{43}H_{53}NO_{15}$: C, 62.69; H, 6.48; N, 1.70. Found: C, 62.49; H, 6.48; N, 1.54.
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