Structure–Activity Relationships of New Taxoids Derived from 14β-Hydroxy-10-deacetylbaccatin III

Iwao Ojima,*,† Young Hoon Park,† Chung-Ming Sun,† Ivana Fenoglio,† Giovanni Appendino,‡ Paula Pera,§ and Ralph J. Bernacki*,§

Department of Chemistry, State University of New York at Stony Brook, Stony Brook, New York 11794-3400, Dipartimento di Scienza e Tecnologia del Farmaco, Università di Torino, 10125 Torino, Italy, and Department of Experimental Therapeutics, Grace Cancer Drug Center, Roswell Park Cancer Institute, Elm and Carlton Streets, Buffalo, New York 14263

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Taxol (paclitaxel), a complex diterpene isolated from the bark of the western yew (*Taxus brevifolia*), is currently considered the most exciting lead in cancer chemotherapy.[1-3] Taxotere (docetaxel), a semisynthetic analog, also has shown great promise.[4] Paclitaxel and docetaxel possess high cytotoxicity and strong antitumor activity against different cancers which have not been effectively treated by existing antitumor drugs.[5,6] Paclitaxel was 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 and lung cancers.[2] Docetaxel is currently in phase II and III clinical trials in United States, Europe, and Japan.[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 multidrug 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.

Recently, a novel taxane diterpenoid, 14β-hydroxy-10-deacetylbaccatin III (14β-OH-DAB), was isolated from the needles of *Taxus wallichiana Zucc.* and other plant parts.[8] Because of an extra hydroxyl group at the C-14 position, 14β-OH-DAB has proven to possess much higher water solubility than the usual 10-deacetylbaccatin III (DAB), which is currently used for the practical production of paclitaxel and docetaxel as mentioned above. Therefore, the new antitumor taxanes derived from 14β-OH-DAB can be expected to have substantially 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.

Syntheses of New Taxoids. A series of new taxoids were synthesized from 14β-OH-DAB using a highly efficient and practical coupling protocol based on the β-lactam synthon method developed in our laboratory.[9-11] Thus, the C-13 side chain precursors, (3*R*,4*S*)-1-acyl-3-(EEO)-4-phenylazetidin-2-ones 1 (EE = ethoxyethyl) with extremely high enantiomeric purity, were obtained through our efficient chiral ester enolate–imine cyclocondensation method[10,11] in four steps in 78–80% overall yields. The 7,10-DiTroc-14β-OH-DAB (**3**) (Troc = 2,2,2-trichloroethoxycarbonyl) were prepared by reacting with 4 equiv of Troc-Cl in pyridine at 80 °C for 5 min in 55% yield (Scheme 1). In a similar manner, 7,10-diTroc-14β-OH-DAB-1,14-carbonate (**2**) was obtained in 75% yield by reacting 14β-OH-DAB with 6 equiv of Troc-Cl in pyridine at 80 °C for 5 min (Scheme 1).

The reaction of **3** with β-lactam **1a** (1.2 equiv) was carried out in the presence of 1.2 equiv of NaHMDS at −40 °C for 30 min to give 7,10-ditroc-2′-EE-14-hydroxydocetaxel-1,-14-carbonate (**4a**) in 86% yield (Scheme 2). The coupling product **4a** was deprotected under modified Commerçon conditions[12] by treating with activated Zn in acetic acid and methanol at 40 °C for 9 h to give 14-hydroxydocetaxel-1,14-carbonate (**5a**) in 70% yield (Scheme 2).[13] 14β-Hydroxypaclitaxel-1,14-carbonate (**5b**) was obtained in the same manner through the coupling of **2** with β-lactam **1b** (1.2 equiv) (89% yield) followed by deprotection (70% yield) (Scheme 2).[13]

The coupling of **3** with β-lactam **1a** and subsequent deprotection were performed under similar conditions to give *pseudo*-docetaxel (**7a**) in 50% overall yield (Scheme 3).[13] In the same manner, 10-deacetyl-*pseudo*-paclitaxel (**7b**) was synthesized through the coupling of β-lactam **1b** with **3**, followed by deprotection in 52% overall yield (Scheme 3).[13]

Cytotoxicity Assay. Cytotoxicities of these new taxanes **5a,b** and **7a,b** were evaluated *in vitro* against human cancer cell lines. As shown in Table 1, these new taxanes possess strong cytotoxicities against human breast, non-small cell lung, ovarian, and colon cancer cells, and especially, **5a** exhibits activity better than that of paclitaxel for non-small cell lung cancer (A549) and colon cancer (HT-29) cell lines. This agent also shows substantial activity against an adriamycin-resistant breast cancer (MCF7-R) cell line. Attachment of an *N*-acylphenylisoserine side chain at C-14 instead of the original C-13 position results in a ca. 10-fold decrease in cytotoxicity. However, **7a** still retains 10 nM level (IC_{50}) cytotoxicities against human cancer cells. This observation might provide us with a very important clue for understanding of the active conformation of paclitaxel series anticancer taxanes. For instance, the molecular modeling of **7a**, starting from the X-ray structure of docetaxel,[15] using SYBYL 6.0 program (Figure 1) reveals an unexpected excellent overlap of the energy-minimized structure of **7a** with that of docetaxel in which the two hydrophobic substituents, i.e., phenyl at C-3′ and *tert*-butoxy group, have exchanged their positions almost perfectly. This implies that the *pseudo*-taxoid **7a** can mimic, to some extent, the binding conformation of docetaxel to the tubulin receptor although the identification of the "active conformation" of paclitaxel and docetaxel should await further investigation.

The microtubule disassembly inhibitory activities of these new taxoids were also examined. The activities relative to paclitaxel are as follows (T = IC_{50} of paclitaxel): **5a**, 0.9T; **5b**, 3T; **7a**, >50T; **7b**, >100T.

The *in vivo* antitumor activity of **5a** has been evaluated against A151 human ovarian carcinoma xenograft in nude athymic mice, which shows an equivalent or slightly better activity than paclitaxel, causing total regression of the tumor. The results of the *in vivo* antitumor activities of these new taxoids will be reported elsewhere in the near future. Further investigations on the SAR of new taxoids derived from 14β-OH-DAB are actively under way.

† State University of New York at Stony Brook.
‡ Univesità di Torino.
§ Roswell Park Cancer Institute.

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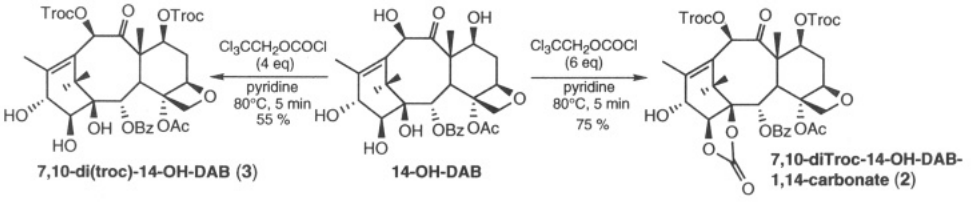
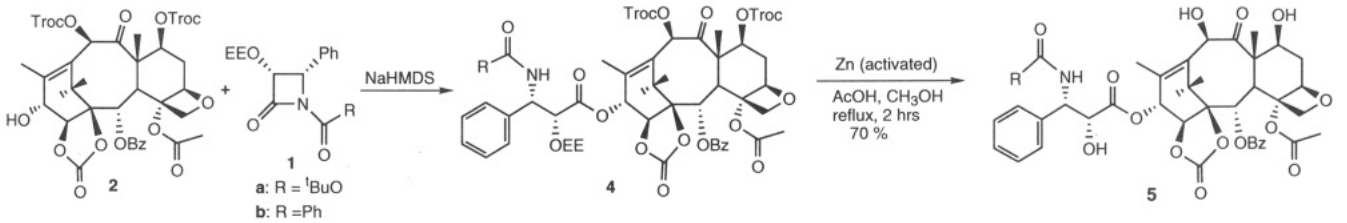
Scheme 1



Scheme 2



Scheme 3

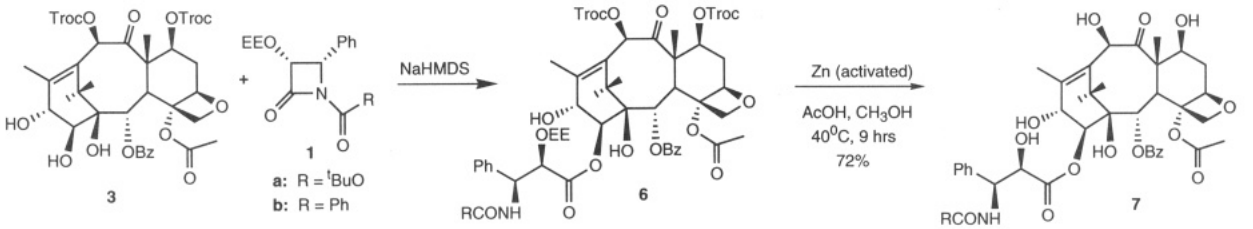


Table 1. Cytotoxicities of New Taxanes (IC_{50}, nM)[a]

cell line	paclitaxel	5a	5b	7a	7b
A121 (ovarian)	6.1 ± 2.4	6.2 ± 0.4	105	80	1044
A549 (NSCL)	3.6 ± 1.1	2.1 ± 0.7	33	31	512
HT-29 (colon)	3.2 ± 0.6	1.8 ± 0.7	24	50	248
MCF7 (breast)	1.7 ± 0.4	1.8 ± 0.8	11	26	269
MCF-7-R (breast)	299 ± 97	543 ± 145	>1000	>1000	>1000

[a] The concentration of compound which inhibit 50% (IC_{50}) of the growth of human tumor cell line, A121 (ovarian carcinoma), A549 (non-small cell lung carcinoma), HT-29 (colon carcinoma), MCF7 (mammary carcinoma), or MCF7-R (mammary carcinoma cells 180-fold resistant to adriamycin), after 72 h drug exposure according to the method developed by Skehan et al.[14] The data represent the mean values of at least three separate experiments.

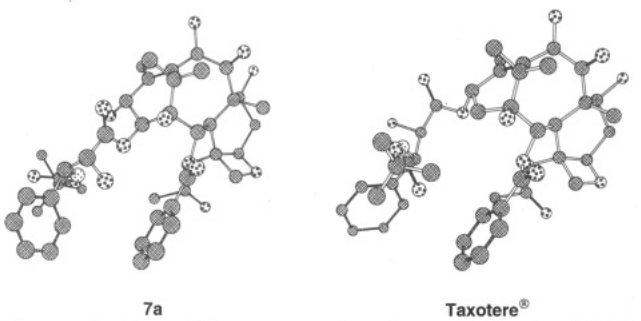


Figure 1. Chem 3D representation of *pseudo*-docetaxel (**7a**) and docetaxel.

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Supplementary Material Available: General experimental procedures for the syntheses of **5a**, **5b**, **7a**, and **7b** from 14-hydroxybaccatin III and the characterization data for new taxoids (7 pages). Ordering information is given on any current masthead page.

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(13) **5a:** mp 191–192 °C; $[\alpha]^{20}_D$ −22.83° (*c* 0.193, $CHCl_3$). **5b:** mp 208 °C dec; $[\alpha]^{20}_D$ −19.0° (*c*, 0.50, $CHCl_3$). **7a:** mp 152–153 °C; $[\alpha]^{20}_D$ +20.9° (*c* 0.67, CH_2Cl_2). **7b:** mp 198–202 °C; $[\alpha]^{20}_D$ −13.2° (*c* 0.38, MeOH). For full identification data, see the supplementary material.

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