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SYNTHESIS AND BIOLOGICAL ACTIVITY OF 3'-ALKYL- AND 3'-ALKENYL-3'-DEPHENYLDOCETAXELS

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Abstract. 3'-Alkyl- and 3'-alkenyl-3'-dephenyldocetaxels are synthesized from 10-deacetylbaccatin III based on the β-lactam synthon method 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. The 3'-isobutenyl, 3'-crotyl, and 3'-isobutyl analogs possess very strong cytotoxicity as well as antitumor activity *in vivo*. 3'-Isobutenyl- as well as 3'-crotyl-3'-dephenyl-10-acetyldocetaxel shows ca. 20 times stronger activity against an adriamycin-resistant human breast cancer cell line (MCF7-R) than those of docetaxel and paclitaxel.

Taxol® (paclitaxel) is currently considered the most exciting lead in cancer chemotherapy.[1-3] Taxotere® (docetaxel), a semisynthetic analog, is also exceptionally 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 for the treatment of advanced ovarian cancer (December, 1992) and breast cancer (April, 1994), and is currently in phase II and III clinical trials for lung, neck and other cancers.[2] Docetaxel is currently in phase II and III clinical trials for breast, lung and other cancers in the United States, Europe, and Japan and expected to be on the market shortly.[6] Recent reports on clinical trials of paclitaxel and docetaxel, however, have disclosed that these highly effective drugs have a number of undesired side effects and are inactive against certain tumor types.[7a] Therefore, it is very important to develop new anticancer drugs which have less undesirable side effects and activity spectra against various tumor types different from those of these two drugs. Paclitaxel and docetaxel are, for example, inactive against colon cancer and renal cell carcinoma,[7a] which are originated from tissues that express constitutively the MDR1 gene.[7b] Thus, the observed lack of activity against these tumors could be partly due to the fact that these two drugs are subjected to multi-drug resistance (MDR)[7c]. Accordingly, it would be worthwhile to develop new taxoids that are not or minimally recognized by cancer cells expressing the MDR phenotype.

In the course of our structure-activity relationship (SAR) study of paclitaxel and docetaxel,[8] we synthesized a series of 3'-cyclohexyl and/or 2-hexahydro analogs and found the fact that 3'-phenyl is not essential for the biological activity of paclitaxel and docetaxel at least *in vitro*.[9] This discovery prompted us to synthesize a series of 3'-alkyl and 3'-alkenyl analogs of docetaxel and look at their structure-activity relationships.[10]

The new 3'-alkyl and 3'-alkenyl analogs of docetaxel were synthesized from 10-deacetylbaccatin III (DAB) based on the β-Lactam Synthon Method using 1-^{t}BOC-(3*R*,4*S*)-4-alkyl-3-hydroxyazetidin-2-ones (**1a,b**) and 1-^{t}BOC-(3*R*,4*S*)-4-alkenyl-3-hydroxyazetidin-2-ones (**2a,b,c**), respectively, as the key precursors of the C-

13 side chains.[12] These 4-alkyl- and 4-alkenyl-β-lactams were readily obtained through efficient chiral ester enolate – imine cyclocondensation in the same manner as reported previously from these laboratories.[11]

The couplings of β-lactams **1** and **2** with 7,10-ditroc-DAB and 7-TES-baccatin III were carried out based on our protocol[12] using NaHMDS as the base followed by deprotection to give the corresponding 3'-alkyl- and 3'-alkenyl-3'-dephenyldocetaxels in good overall yields (Chart 1).[13] We also synthesized 3'-isobutenyl-3'-dephenylpaclitaxel (**RAH-1**)[10] in a similar manner for comparison purpose.

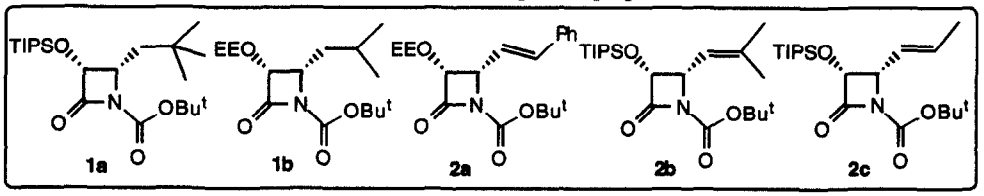
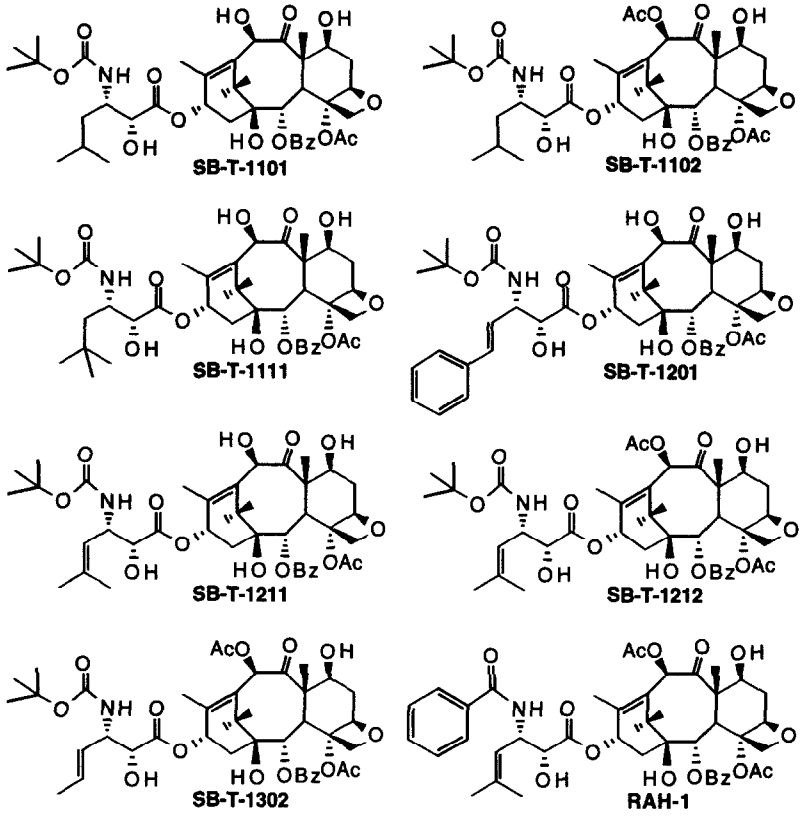


Chart 1



Microtubule Disassembly Inhibitory Activity and Cytotoxicity of the 3'-Alkyl and 3'-Alkenyl Analogs. Biological activities of four 3'-alkyl and 3'-alkenyl analogs of docetaxel were evaluated in three assay systems, *i.e.*, inhibition of microtubule disassembly and cytotoxicity against murine P388 leukemia cell line as

well as doxorubicin-resistant leukemia cell line (P388/Dox) which is a MDR expressing cell line cross resistant to paclitaxel and docetaxel.[14] Results are listed in Table 1.[12] As Table 1 shows, the 3'-isobutyl analog **SB-T-1101** and the 3'-isobutenyl analog **SB-T-1211** possess excellent activities comparable to docetaxel. It is also obvious that the activity is very sensitive to the bulkiness of the 3'-substituents.

Table 1. Microtubule Disassembly Inhibitory Activity and *in vitro* Cytotoxicity of 3'-Alkyl and 3'-Alkenyl Analogs

Taxoid	Microtubule disassembly inhibitory activity[a] IC_{50}/IC_{50}(paclitaxel)	P388 cell line[b] IC_{50} (nM)	P388/Dox[b] IC_{50} (μM)
Docetaxel	0.70T	9.9	1.86
SB-T-1101	0.78T	12.2	1.59
SB-T-1201	1.45T	264	7.49
SB-T-1111	1.45T	125	6.88
SB-T-1211	0.64T	12.8	2.36

[a] IC_{50} represents the concentration of an agent leading to 50% inhibition of the rate of microtubule disassembly. IC_{50}(paclitaxel) is the IC_{50} value of paclitaxel in the same assay. In the same assay, the IC_{50} of paclitaxel is 0.015 mM. [b] IC_{50} represents the concentration that inhibits 50% of cell proliferation.

The cytotoxicity of 3'-alkyl and 3'-alkenyl analogs of docetaxel and a 3'-alkenyl paclitaxel analog **RAH-1** were evaluated against human ovarian (A121), human non-small cell lung (A549), human colon (HT-29), and human breast (MCF7) cancer cell lines. In addition to these cell lines, the activity against an adriamycin-resistant human breast cancer cell line (MCF7-R) was also examined. Results are summarized in Table 2.[13] As Table 2 shows, 3'-isobutenyl, 3'-crotyl, and 3'-isobutyl analogs, especially the 3'-isobutenyl and 3'-crotyl analogs, **SB-T-1212** and **SB-T-1302**, exhibit excellent cytotoxicity. It is noteworthy that **SB-T-1102**, **SB-T-1212**, **SB-T-1302** show one order of magnitude better activity than paclitaxel and docetaxel against MCF7-R. This finding may provide significant information for the development of newer antitumor agents effective against tumors expressing the MDR phenotype.

Table 2. Cytotoxicities (IC_{50} nM)[a] of 3'-Alkyl and 3'-Alkenyl Analogs

Taxoid	A121[a] (ovarian)	A549[a] (NSCLC)	HT-29[a] (colon)	MCF7[a] (breast)	MCF7-R[a,b]
Paclitaxel	6.1	3.6	3.2	1.7	300
Docetaxel	1.2	1.0	1.2	1.0	235
RAH-1	1.4	0.45	0.96	0.54	113
SB-T-1101	1.9	0.70	0.50	0.80	107
SB-T-1102	3.8	0.98	3.2	4.0	**36**
SB-T-1212	0.46	0.27	0.63	0.55	**12**
SB-T-1302	0.90	0.54	0.76	0.51	**14**

[a] See the footnote of Table 1. [b] MCF7-R = mammary carcinoma cells 180 fold resistant to adriamycin.

Antitumor Activity of the 3'-Alkyl and 3'-Alkenyl Analogs *in vivo*. The *in vivo* antitumor activity of **SB-T-1101** and **SB-T-1211** was evaluated against B16 melanoma in B6D2F1 mice. Taxoids (0.4 mL/mouse) were administered intra-venously (i.v.) on days of 5, 7, and 9 (see Ref. 9 for details). Results are as follows: SB-T-**1101**, T/C = 5% (20 mg/Kg/day), time for median tumor to reach 1,000 mg (days) = 26.03, $\log_{10}$ cell kill = 1.97; **SB-T-1211**, T/C = 8% (12.4 mg/Kg/day), time for median tumor to reach 1,000 mg (days) = 27.54, $\log_{10}$ cell kill = 2.25. The results clearly indicate that both analogs are very active *in vivo*, and their activities are equivalent to that of docetaxel in the same assay.

Consequently, it appears that 3'-alkyl and 3'-alkenyl analogs of docetaxel and paclitaxel have opened a new avenue for the development of newer and potent taxoid antitumor agents.

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