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SYNTHESIS AND BIOLOGICAL ACTIVITY OF NOVEL 3'-TRIFLUOROMETHYL TAXOIDS

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Abstract. Second generation taxoids possessing a trifluoromethyl moiety in place of the 3'-phenyl group are synthesized by means of the *β-Lactam Synthon Method.* The in vitro cytotoxicities of these new taxoids are evaluated against several different human cancer cell lines and found to exhibit greatly enhanced activities as compared to those of paclitaxel and docetaxel. The activity enhancement is most remarkable against a drug-resistant breast cancer cell line, MCF7-R, expressing MDR phenotype.

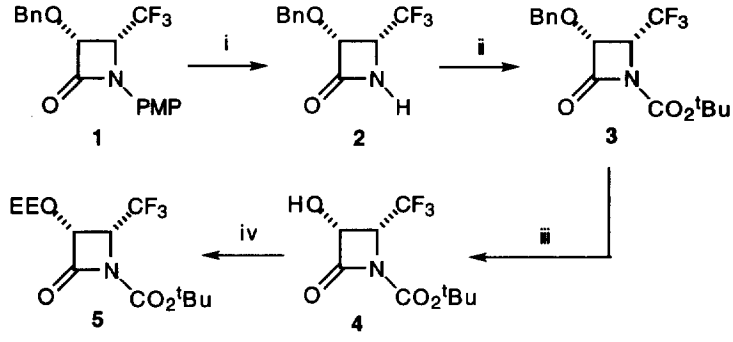
Over the past several years, both Taxol® (paclitaxel)[1] and Taxotere® (docetaxel)[2] have been proven to be the most exciting leading drugs in the fight against cancers.[3-7] Through their unique mode of action,[8-10] these two drugs exhibit impressive activity against various types of cancer that have not been effectively treated by other conventional anticancer agents. Paclitaxel has obtained FDA approval for the treatment of advanced ovarian cancer (December 1992) and breast cancer (April 1994) while docetaxel has recently been approved for the treatment of breast cancer (May 1996). Clinical trials for other cancers as well as combination therapy are currently in progress.[3]

Although these two drugs have made great strides in the treatment of various cancers, especially breast and ovarian cancers, reports have indicated that their use often results in a number of undesirable side effects.[11] These side effects, along with the onset of multidrug resistance (MDR), clearly indicate the necessity of developing the second generation taxoid anticancer drugs having fewer side effects and much improved activity against drug-resistant tumors.

In the course of our systematic structure-activity relationship studies on taxoids, we designed a new series of fluorine-containing taxoids[12,13] bearing a trifluoromethyl moiety at the C-3' position in place of the phenyl group. These new 3'-CF_3-taxoids were synthesized through the coupling of racemic 1-^{t}Boc-3-(1-ethoxyethoxy)-4-trifluoromethylazetidin-2-one (**5**) and 7-TES-baccatins **6**.

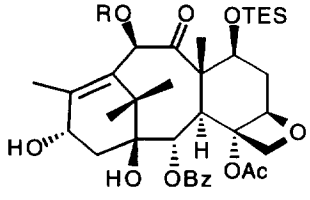
Racemic β-lactam **1** was prepared via a ketene-imine [2+2] cycloaddition, following the procedure reported by Abouabdellah et al.[14] with modifications. The *p*-methoxyphenyl (PMP) group of β-lactam **1** was oxidatively cleaved by cerium ammonium nitrate (CAN) and the resulting NH free β-lactam **2** was reacted with (^{t}Boc)$_2$O in the presence of 4-dimethylaminopyridine (DMAP) and triethylamine (TEA) to afford β-lactam **3**. The β-lactam **3** was subjected to hydrogenolysis (H_2 and Pd/C in ethyl acetate) to afford 3-hydroxy β-lactam **4**, which was subsequently protected as 1-ethoxyethyl (EE) ether to give β-lactam **5** in quantitative yield for the last two steps. (Scheme 1).

Scheme 1



i) CAN, H_2O/CH_3CN, -5°C, 60%; ii) $(^tBOC)_2O$, CH_2Cl_2, 65%;
iii) H_2, Pd/C, EtOAc, 100%; iv) ethyl vinyl ether, *p*TSA, THF, 100%

7-TES-baccatin III and its C-10 modified analogs were readily prepared from 10-deacetylbaccatin III by applying the previously published methods[15,16] with some modifications.[17] Thus, the C-7 hydroxyl group of 10-deacetylbaccatin III was protected as a triethylsilyl ether first, followed by modification of the hydroxyl group at C-10 with triethylsilyl chloride, acid chlorides, alkyl chloroformates, and *N,N*-dialkylcarbamoyl chlorides using LiHMDS as the base to afford baccatins **6a-i** in 78-92% yield.[17]

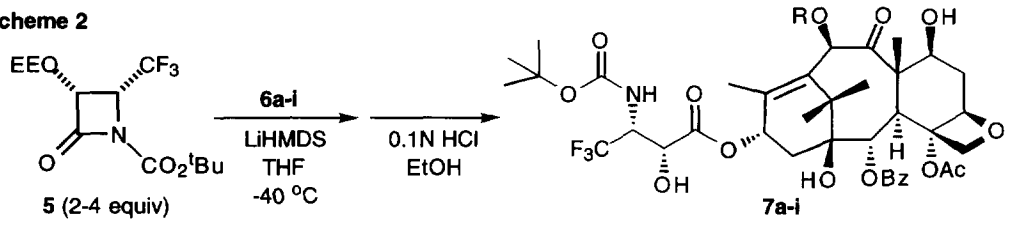


6a: R = TES
6b: R = Ac
6c: R = Me_2N-CO
6d: R = cyclopropane-CO
6e: R = MeO-CO
6f: R = morpholine-4-CO
6g: R = Et-CO
6h: R = $Me(CH_2)_3$-CO
6i: R = Me_3CCH_2-CO

Coupling of the racemic 1-^{t}Boc-β-lactam **5** (excess) with 7-TES-baccatins **6** was carried out using the coupling protocol developed in these laboratories with expectation of the occurrence of high level kinetic resolution at low temperatures (Scheme 2). This type of kinetic resolution in the coupling of racemic β-lactam intermediates and metallated baccatin III was previously noted in patent literature by Holton and Biediger.[18,19] However, the examples shown are all for 4-aryl-β-lactams and thus it was not predictable what level kinetic resolution might take place for this particular 4-CF_3-β-lactam **5**. To a solution of baccatin **6** and β-lactam **5** (2-4 equiv) in THF was added 1.5-2.0 equiv of LiHMDS in THF at -40 °C. After reacting for 30 min at this temperature, it was necessary to allow the reaction mixture to warm to -20 °C (and in some cases up to 0 °C) over a period of 1-2 h in order for the reaction to reach optimal conversion (see Table 1). After the conversion of the coupling reaction was checked by ^1H NMR analysis, the reaction mixture was treated with 0.1N hydrochloric acid in ethanol at 0 °C ---> room temperature for 12-16 h, followed by purification by flash chromatography on silica gel to afford 3'-CF_3-taxoids **7** with 9:1 ~ >30:1 diastereomer ratio in fairly good 2-step

yields. The ratios of two diastereomers were determined by ^{19}F NMR analyses of the final taxoids **7a-i**. Results are summarized in Table 1.

Scheme 2



As Table 1 shows, a high to excellent level kinetic resolution of racemic N-^{t}Boc-β-lactam **5** is observed in all cases examined. Thus, it can be said that the chiral recognition of (3*R*,4*S*)- and (3*S*,4*R*)-β-lactams **5** by the chiral metal alkoxide of baccatin **6** is efficient enough to yield taxoid **7** with desired configurations at the C-2' and C-3' positions as the predominant product. In the reactions of **6a** (R = TES), **6b** (R = Ac) and **6c** (Me_2N-CO) with **5**, (2'*R*,3'*S*)-taxoids **7a**, **7b**, and **7c**, respectively, were exclusively obtained. Also, the reaction of **6g** (R = Et-CO) with **5** gave **7g** with >30:1 diastereomer ratio. Although attempts to separate out minor diastereomers, i.e., (2'*S*,3'*R*)-taxoids, by column chromatography have not been successful, the satisfactory elemental analyses and the close pairing of ^1H NMR as well as ^{13}C NMR spectra of the taxoids **7d-i** make it quite reasonable to assume that the minor products in **7d-i** are the (2'*S*, 3'*R*)-diastereomers arising from the coupling of (3*S*,4*R*)-enantiomer of the β-lactams **5** with the baccatins **6d-i**. As Table 1 shows, the two-step yields of **7** are only fairly good, but the conversion of the coupling is high to extremely high. This implies that decomposition of baccatin **6** or unidentified side reaction(s) might have occurred during the kinetic resolution – coupling process. Thus, optimization of the process is currently underway.

Table 1. Syntheses of 3'-CF_3-taxoids **7** through the coupling of β-lactams **5** with baccatins **6**.

Taxoid	R	Reaction Temp. (°C)	Conversion of Coupling (%)[a]	Yield (%)[b]	Isomer Ratio[c] (2'*R*,3'*S*): (2'*S*,3'*R*)
7a	H	-40 to -20	80	54 (67)	single isomer
7b	Ac	-40 to -10	72	41 (57)	single isomer
7c	Me_2N-CO	-40 to 0	100	63	single isomer
7d	cyclopropane-CO	-40 to 0	100	64	10:1
7e	MeO-CO	-40 to -20	93	54 (58)	24:1
7f	morpholine-4-CO	-40 to -20	100	60	23:1
7g	Et-CO	-40 to -15	100	74	>30:1
7h	$CH_3(CH_2)_3$-CO	-40 to -10	100	56	9:1
7i	$(CH_3)_3CCH_2$-CO	-40 to -20	91	59 (65)	22:1

[a]Based on the consumed baccatin **6**. [b]Two-step yield. Values in parentheses are conversion yields. [c]Determined by ^{19}F NMR analysis of taxoids **7a-i**.

The cytotoxicities of new 3'-CF_3-taxoids **7** thus obtained were evaluated against several human cancer cell lines, A121 (ovarian carcinoma), A549 (non-small cell lung carcinoma), HT-29 (colon carcinoma), MCF-7 (mammary carcinoma), and MCF7-R (mammary carcinoma 180-fold resistant to doxorubicin, expressing MDR phenotype). Results are summarized in Table 2. As Table 2 shows, 3'-CF_3-taxoids **7a-i** possess excellent activity against these human cancer cell lines. In virtually every case, these new taxoids are substantially more active than either paclitaxel or docetaxel. However, the most remarkable results are their *one order of magnitude* better activities as compared to paclitaxel and docetaxel against the drug-resistant breast cancer cell line, MCF7-R.

Table 2. Cytotoxicity (IC_{50} nM)[a] of 3'-CF_3-taxoids

Taxoid	A121 (ovarian)	A549 (NSCLC)	HT-29 (colon)	MCF-7 (breast)	MCF7-R (breast)
Paclitaxel	6.3	3.6	3.6	1.7	299
Docetaxel	1.2	1.0	1.2	1.0	235
7a	1.15	0.44	0.65	0.44	156
7b	0.3	0.2	0.4	0.6	**17**
7c	0.3	0.2	0.4	0.3	**21**
7d	0.4	0.4	0.5	0.5	**16**
7e	0.3	0.2	0.4	0.4	**21**
7f	0.5	0.4	0.4	0.4	**48**
7g	0.3	0.3	0.4	0.3	**14**
7h	0.7	0.8	1.4	0.7	**26**
7i	0.5	0.5	0.6	0.5	**12**

[a] The concentration of taxoid that inhibits 50% of the growth of human tumor cells after 72 h drug exposure.[20]

The comparison of C-10 free taxoid **7a** (R = H) with C-10–acylated taxoids **7b-i** clearly indicates that the observed remarkable activity enhancement against the drug-resistant breast cancer cell line, MCF7-R, is attributed to the acylation at the C-10 position, while this modification has little effect on the activity against the normal cancer cell lines, i.e., there is no discernible relationship between the activity against the normal cancer cell lines and that against the drug-resistant cell line. Similar effects of C-10 acylation were observed in the 3'-alkyl and 3'-alkenyl series of taxoids developed in these laboratories, in which the activity against MCF7-R is very sensitive to the bulkiness of the C-10 modifier.[17] In these 3'-CF_3-taxoids, however, the activity is not so sensitive to the steric bulk of the C-10 modifier, e.g., **7h** (R = 3,3-dimethylbutanoyl) and **7g** (R = Et-CO) exhibit virtually the same level of activity. We suspect that the C-10 modifier is playing a key role in suppressing the binding of the taxoids to P-glycoprotein which has been show to be responsible for MDR[21] although this modification does not appear to interfere with binding to tubulin.

As a part of our ongoing investigation on the bioactive conformation(s) of paclitaxel, docetaxel, and various taxoids, we have been successfully using "fluorine probes", which enable us to use ^{19}F NMR analyses, especially variable temperature (VT) experiments, to look at dynamic features of these molecules.[13] The

conformational analyses of paclitaxel, docetaxel and their analogs have revealed the existence of "hydrophobic collapse"[22] or "hydrophobic clustering"[13,23] conformations in aqueous media, e.g., DMSO-D_2O, in that the phenyl group of 2-benzoate, the phenyl group at C-3', and the methyl group of 4-acetoxy moiety form a hydrophobic cluster in contrast to the conformation observed in aprotic media, e.g., $CDCl_3$ or CD_2Cl_2.[22,24-26] We were particularly interested in whether CF_3-taxoids take the "hydrophobic clustering" conformation in DMSO-D_2O and in MeOD using **7b** as the representative. Surprisingly, **7b** does not show different conformations in DMSO-D_2O, MeOD, and CD_2Cl_2 based on VT experiments on its ^{19}F chemical shifts as well as $J_{2'\text{-}H,3'\text{-}H}$ coupling constant. The latter value directly reflects the dihedral angle of $H^{2'}\text{-}C^{2'}\text{-}C^{3'}\text{-}H^{3'}$, from which the most likely conformation of the whole molecule is deduced using molecular modeling (SYBYL and Biosym programs). The observed $J_{2'\text{-}H,3'\text{-}H}$ coupling constant was ca 2.0 Hz (corresponds to ca 60° for the $H^{2'}\text{-}C^{2'}\text{-}C^{3'}\text{-}H^3$ dihedral angle) in all three solvents and it did not show any detectable temperature dependence. *This means that this molecule does not form "hydrophobic cluster" even in DMSO-D_2O.* The Chem 3D representations of the most likely solution conformation of **7b** are shown in Figure 1. This conformation is similar to the X-ray structure of docetaxel although the 3'-phenyl is replaced by a sterically and electronically very different CF_3 group. The fact that **7b** and related CF_3-taxoids are extremely active warrants further investigation on the interaction of this molecule with tubulin using the CF_3 group as a probe.

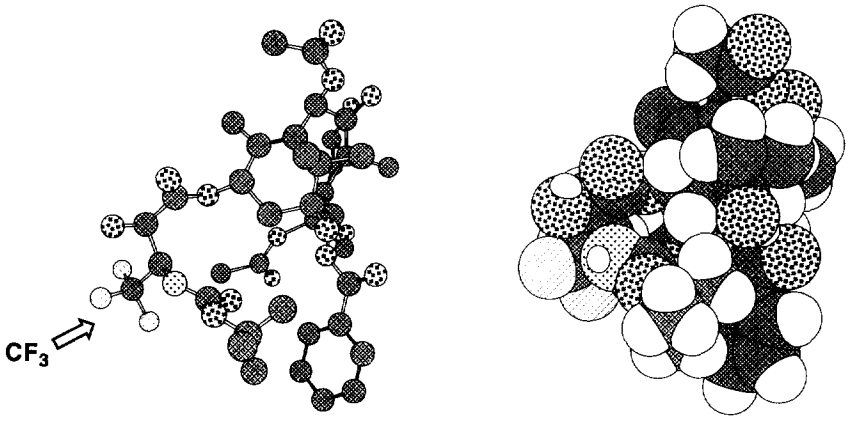


Figure 1. Chem 3D representations of the solution structure of **7b**

Further studies on the development of potent second-generation taxoids as well as the bioactive conformations of taxoid antitumor agents using "fluorine probes" are actively underway.

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