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Recent Advances in the Chemistry and Biology of New Generation Taxoids[‡]

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Abstract

Among the numerous chemotherapeutic drugs, paclitaxel and docetaxel are among the most widely used against various types of cancer. However, these drugs cause undesirable side effects as well as drug resistance. Therefore, it is essential to develop “taxane” anticancer agents with better pharmacological properties and improved activity especially against drug-resistant cancers. Several laboratories have performed extensive SAR studies on paclitaxel. Our SAR studies have led to the development of numerous highly potent novel second- and third-generation taxoids with systematic modifications at the C-2, C-10 and C-3' positions. The third-generation taxoids showed virtually no difference in potency against drug-resistant and drug-sensitive cell lines. Some of the new generation taxoids also exhibited excellent cytotoxicity against pancreatic cell lines expressing multidrug-resistant genes. We have also designed taxoids with strategic fluorine incorporation to investigate their effects on the cytotoxicity and the blockage of known metabolic pathways. Furthermore, we have successfully employed computational biology analysis to design novel macrocyclic taxoids to mimic the bioactive conformation of paclitaxel. This account describes our work on the design, synthesis and biological evaluation of these novel taxoids, which has led to the discovery of very promising candidates for further preclinical studies.

Introduction

Cancer is one of the leading causes of death in the world and is the leading cause of death for people under the age of 85 in the U.S.^{1,2} Paclitaxel and docetaxel are among the most widely used chemotherapeutic drugs especially against some types of cancer like ovarian, breast, lung, and Kaposi's sarcoma.^{3,4} These anticancer agents bind to the β -tubulin subunit of the tubulin dimer and accelerate their polymerization resulting in stabilized microtubules. This causes the arrest of the cell division cycle mainly at the G2/M phase resulting in apoptosis through the cell-signaling cascade.^{5,6} Despite their potent antitumor activity, paclitaxel and docetaxel cause undesirable side effects as well as drug resistance.³ Thus, it is essential to develop new taxane anticancer agents that will have fewer side effects, enhanced activity against drug-resistant human tumors, and superior pharmacological properties.

Our SAR study on taxoids has indicated that (i) the C-3'-phenyl group can be replaced with an alkenyl or alkyl group and (ii) the C-10 position can be modified with certain acyl groups that make the compounds one-two orders of magnitude more potent than the parent drugs (paclitaxel and docetaxel) against drug-resistant human breast cancer cell lines. These highly potent taxoids were termed “second-generation taxoids”.⁷ We have also found that substitution

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(e.g., MeO, N₃, Cl, F, etc.) at the *meta* position of the C-2-benzoyl group of the second-generation taxoids enhanced their activities compared to the parent drugs against drug-resistant human breast cancer cell lines.^{8,9} Multidrug resistance to paclitaxel is caused mainly by the overexpression of ABC transporters, e.g., Pgp,¹⁰ but there are other mechanisms of drug resistance like the overexpression of specific tubulin isotypes.¹¹⁻¹⁴ The microtubules with altered β -tubulin isotype compositions respond differently to paclitaxel.¹⁵ Recently, Ferlini has reported that the C-seco-taxoid IDN 5390 is eight-fold more active than paclitaxel against drug-resistant and paclitaxel-resistant cell lines.¹⁶⁻¹⁸ We have synthesized a series of IDN 5390 analogues with C-2-benzoate modifications at the *meta* position to investigate their effects on cytotoxicity.

Fluorine is an important heteroatom used in drug design¹⁹ because of its favorable atomic properties that result in higher metabolic stability, often increased binding to target molecules, and increased lipophilicity and membrane permeability among other properties in biologically active compounds. Accordingly, we have synthesized fluoro-taxoids to investigate the effects of fluorine-incorporation on the cytotoxicity and the blockage of known metabolic pathways.²⁰⁻²³

We have also designed novel macrocyclic paclitaxel congeners to mimic the bioactive conformation of paclitaxel based on computational analysis.²⁴ This account describes our work on the synthesis and biological evaluation of new-generation taxoids bearing various substituents at the C-2, C-10, C-3' and C-3' N positions as well as macrocyclic taxoids.

Synthesis of C-10-Modified Taxoids

Second-generation taxoids such as **6**, bearing various C-10 aromatic acyl groups, were synthesized from 10-deacetylbaccatin III (DAB, **1**), by applying the procedure developed by Georg²⁵ via 7-TES-baccatin III **2**, Ojima-Holton coupling^{7,26} of **2** with *N*-*t*-Boc- β -lactam **3a**,^{27,28} deacetylation, C-10-acylation, and deprotection by HF-pyridine (Schemes 1 and 2; Table 1).²⁹

Synthesis of C-2- and C-3'- Modified Taxoids

Second-generation taxoids **12** and **13** (Table 3), with different substituents at the *meta* position of the C-2 benzoyl group, were synthesized through the Ojima-Holton coupling^{7,30} of baccatins **11a-l** (Scheme 3; Table 2) with β -lactams **3a-f**, as illustrated in Scheme 4.²⁹ Baccatins **11a-l** were synthesized via 7,10,13-tris-TES-2-debenzoyl-DAB **8**.^{31,32} C-2-modified tris-TES-DABs **9a-f**, 7-TES-2-modified DABs **10a-f**, and selective acylation at the C-10 position of **10a-f** (Scheme 3; Table 2). Enantiopure β -lactams **3a-f** with various C-4 substituents were prepared through efficient chiral ester enolate-imine cyclocondensations^{7,9,26,28,30,33} or [2+2] ketene-imine cycloaddition, followed by enzymatic optical resolution.³⁴ Taxoid **13g** was obtained by hydrogenation **12g** on Pd/C (Scheme 4).

Synthesis of C-3'N Modified Taxoids

C-3'N-modified taxoids **15** and **16** were synthesized using enantiopure β -lactams bearing various *N*-acyl or *N*-carbalkoxy groups (Scheme 5, Table 4).²⁹ These β -lactams were prepared by reacting *NH*-free 3-TBSO- or 3-TIPSO- β -lactam with acid chlorides or chloroformates. The resulting β -lactams **14a-h** were coupled with baccatin **11b** followed by deprotection of silyl groups to afford the desired taxoids **15a-l**. Hydrogenation on Pd/C of selected **15** gave the corresponding taxoids **16** in nearly quantitative yields.

Synthesis of C-3'-Difluoromethyl- and C-3'-Trifluoromethyl-taxoids

A series of the second-generation taxoids **21** and **22** with C-3'-CF₂H- and C-3'-CF₃- groups, respectively, were synthesized from β -lactams **17** (Rf = CF₂H or CF₃) and baccatins **18** by means of the β -Lactam Synthon Method,³⁵ as shown in Scheme 6.³⁶

Synthesis of C-2-(3-Fluorobenzoyl)-C-seco-taxoids

Recently, a C-seco-taxoid, IDN5390 (Figure 1), was reported to exhibit several times better potency than paclitaxel against drug-resistant ovarian cancer cell lines overexpressing the class III tubulin isotype.¹⁶ As a part of our SAR study on IDN5390, we investigated two fluorine-containing analogs, SB-T-10104 (**28a**) and SB-T-10204 (**28b**) (Scheme 7). These two C-seco-fluorotaxoids, **28a** and **28b**, were synthesized through the Ojima-Holton coupling of 7,9-di-TES-2-(3-fluorobenzoyl)-C-seco-baccatin **25** with β -lactams **26a**²⁸ and **26b**,²⁷ respectively. Di-TES-C-seco-baccatin **25** was prepared from 2-(3-fluorobenzoyl)-10-deacetylbaccatin **23**³⁷ using Appendino's protocol^{38,39} as follows: (a) Baccatin **23** was oxidized with Cu(OAc)₂ and air to give the corresponding 10-oxo-baccatin **24**, which was then treated with L-selectride, followed by TES protection, to afford di-TES-C-seco-baccatin **25** (Scheme 7).

Synthesis of C-3'-Difluorovinyl-taxoids

Our recent metabolism studies on 3'-isobutyl- and 3'-isobutenyl-taxoids has disclosed that the metabolism of second-generation taxoids (SB-T-1214, SB-T-1216, and SB-T-1103) is markedly different from those of docetaxel and paclitaxel.⁴⁰ These taxoids are metabolized by CYP 3A4 of the cytochrome P450 family enzymes primarily at the two allylic methyl groups of the C-3'-isobutenyl group and the methine moiety of the 3'-isobutyl group (Figure 2). This is a sharp contrast from the known result that the *t*-butyl group of the C-3'*N*-*t*-Boc moiety is the single predominant metabolic site for docetaxel.⁴¹ This prompted us to design and synthesize 3'-difluorovinyl-taxoids, to block the allylic oxidation by CYP 3A4, which should enhance the metabolic stability and activity in vivo.

The novel (3*R*,4*S*)-1-*t*-Boc-3-TIPSO-4-difluorovinyl- β -lactam **32**(+) was coupled with baccatins **18** (Scheme 8).⁴² The β -lactam **32**(+) was prepared from 4-formyl- β -lactam **29**(+) by the Wittig-type reaction (Scheme 8). The Ojima-Holton coupling^{30,43,44} of β -lactam **32**(+) with modified baccatins **18**³⁷ and the subsequent removal of the silyl protecting groups gave the corresponding C-3'-difluorovinyl-taxoids **33** in good to excellent yields (Scheme 8).⁴²

Design and Synthesis of Novel C-14-C-3'BzN-linked Macrocyclic Taxoids

The first cryo-electron microscopy (cryo-EM) structure of paclitaxel-bound Zn²⁺-stabilized α , β -tubulin dimer (1TUB structure) reported in 1998 at 3.7 Å resolution,⁴⁵ which used the docetaxel crystal structure for display, opened a new era for the structural biology and medicinal chemistry of paclitaxel. The 1TUB structure was later refined to 3.5 Å resolution with a paclitaxel molecule in 2001 (1JFF structure).⁴⁶ However, the resolution of these cryo-EM structures was not high enough to solve the binding conformation of paclitaxel. Thus, a computational study of the electron-density map was performed and the "T-Taxol" conformation was proposed.⁴⁷ To prove the validity of the "T-Taxol" structure, rigidified paclitaxel congeners were designed, synthesized and assayed for their tubulin polymerization ability and cytotoxicity⁴⁸⁻⁵² based on the "T-Taxol" structure. Among those "T-Taxol" mimics, C-4-C-3'-linked macrocyclic taxoids showed higher activities than paclitaxel in the cytotoxicity and tubulin-polymerization assays.^{49,51} We proposed "REDOR-Taxol" as a valid microtubule-bound paclitaxel structure in 2005⁵³ based on the two ¹³C-¹⁹F intramolecular distances of the microtubule-bound 2-(4-fluorobenzoyl)paclitaxel experimentally obtained by means of a REDOR NMR study,⁵⁴ MD analysis of paclitaxel conformers, photoaffinity

labeling,⁵⁵ and molecular modeling studies using the 1TUB coordinate.³⁷ The “REDOR-Taxol” structure was further refined using the 1JFF coordinate. The “REDOR-Taxol (1JFF)” is fully consistent with the additional REDOR-experiments by Schaefer and collaborators⁵⁶ and also accommodates highly active macrocyclic paclitaxel analogs designed based on the “T-Taxol” structure.²⁴ The C-2'-OH group interacts with His²²⁷ as the hydrogen bond donor in the “REDOR-Taxol”,⁵³ while the H-bonding is between the C-2'-OH and the backbone carbonyl oxygen of Arg³⁶⁹ in the “T-Taxol”.⁴⁷ Accordingly, we have designed novel macrocyclic taxoids **34a-c** by linking the C-14 and C-3'BzN groups, which mimic the “REDOR-Taxol” structure to examine the level of biological activity compared to that of paclitaxel (Figure 3).²⁴ As the overlays in Figure 4 illustrate, **34a** and **34c** appear to mimic the REDOR-Taxol structure very well, while the C-3'*N*-benzoyl group of **34b** deviates from the rest.²⁴

β -Lactams **37a-d** were prepared in excellent yields through acylation of the corresponding enantiopure N-H free β -lactams with 2-alkenylbenzoyl chlorides (Scheme 9). The 7-TES-14 β -allyloxy-baccatin **40** was prepared by following the method previously reported by us from 14-OH-DAB.⁵³ The Ojima-Holton coupling of **40** with β -lactams **37a-d** gave the corresponding paclitaxel-dienes **41a-d** bearing olefinic groups at the C-14 position as well as the *ortho* position of the C-3'BzN moiety (Scheme 10).²⁴

As Scheme 11 shows, the RCM reactions of paclitaxel-dienes **41a** and **41d** catalyzed by the “1st-generation Grubbs catalyst” proceeded smoothly at room temperature to give the corresponding macrocyclic taxoids **42a** and **42d**, respectively. The subsequent deprotection of all silyl groups with HF-pyridine afforded the designed macrocyclic taxoids **34a** and **34d**, respectively, in high yields.²⁴ In both products, *E*-isomer was formed exclusively. On the other hand, the RCM reaction of **41c**, proceeded slowly and gave a ca. 1:1 mixture of **42c-E** and **42c-Z** isomers, which were separated and deprotected to give **34c-E** and **34c-Z**, respectively, in high yields (Scheme 12).²⁴ The observed low reactivity of **41c** and the lack of stereoselectivity in the alkene formation can be attributed to the larger ring size of the product (s).

In contrast, the RCM reaction of paclitaxel-diene **41b** did not proceed as anticipated and gave **42b** unexpectedly, which was deprotected to afford macrocyclic taxoid **43b** in fairly good yield (Scheme 13).²⁴ The ¹H and ¹³C NMR and 2D NMR analyses suggested that **43b** should possess a butenylene unit between the C-14-O and the *ortho* position of the C-3'*N*-benzoyl moiety and the newly formed double bond should be conjugated to the phenyl group. This proposed structure was confirmed by the X-ray crystallographic analysis of **43b** (SB-T-2054), as shown in Figure 5.²⁴ A plausible mechanism for this Ru-catalyzed novel olefin-olefin coupling reaction was proposed,²⁴ which includes the formation of a regioisomeric metalacyclobutene, its isomerization to the corresponding σ -allylic intermediate, followed by reductive elimination.

Biological Activities of New Generation Taxoids

The new generation taxoids were evaluated for their cytotoxicity against various drug-sensitive and drug-resistant cancer cell lines. Table 5 summarizes the potencies of C-10-modified taxoids **6a–6o** against LCC6-WT and LCC6-MDR cell lines.²⁹ All taxoids exhibit similar or better activities than paclitaxel against LCC6-WT, while more than a half of this series of taxoids show two orders of magnitude better activity than that of paclitaxel against LCC6-MDR. The dramatic decrease in the R/S ratio for a majority of this series of taxoids (R/S ratio at or below 3), which is an excellent indicator of the level of drug-resistance associated with drugs, is the most noteworthy feature. Taxoid **6d** shows almost no difference against drug-resistant and drug-sensitive cell lines with the R/S ratio of 1.2.

Taxoids **6a** and **6d** with a benzoyl group and a 4-methoxybenzoyl group at C-10, respectively, possess highest potencies against LCC6-MDR, while taxoid **6k** with a 4-methoxyphenylacetyl is the least potent but possesses the highest potency against LCC6-WT. Taxoids with a substituted or unsubstituted benzoyl group (**6a-6e**) or 2-phenylpropanoyl group (**6m-6o**) are highly potent against LCC6-MDR, while taxoids with arylacetyl substituents (**6i-6k**) show reduced activity against LCC6-MDR. Elongation of the alkyl chain of **6i-6k** just by one carbon restores high potency against LCC6-MDR. The results suggest that the C-10-substituents are critical to the modulation of Pgp efflux pump in taxoids.

The activities of C-3'N-modified 10-propanoyl-taxoids are summarized in Table 6.²⁹ Most of these C-3'N-modified taxoids possess better potency against LCC6-WT and MCF7 cell lines and one-two orders of magnitude higher potency against drug-resistant LCC6-MDR and NCI/ADR cell lines, as compared with paclitaxel. Taxoids **15d** and **15e** exhibit high potency against these cell lines and possess cytotoxicity comparable to their parent taxoids SB-T-1213⁷ and SB-T-1103.⁷ The results show that the *t*-Boc group at the C-3'N position, which is the “gold standard”, can be replaced by cycloalkenoyl groups without losing potency.

Table 7 summarizes the cytotoxicity assay results of taxoids with a modified C2-benzoyl group at its *meta* position.²⁹ The majority of these new second-generation taxoids (**12** and **13**) with a C-3'N-*t*-Boc group shows remarkable potency against drug-resistant (Pgp+) cancer cell lines, LCC6-MDR and NCI/ADR with R/S ratios less than 3 in many cases and less than 1 in three cases (**12g** for LCC6-WT : LCC6-MDR and MCF7:NCI/ADR as well as **13g** for LCC6-WT : LCC6-MDR). It can be said that the Pgp-mediated MDR is completely circumvented by the new taxoids **12g** and **13g**. *Accordingly, we have defined these new-generation taxoids, which can virtually circumvent the Pgp-mediated MDR, as the “third-generation” taxoids.*

The potency decreases in the order: F > Cl > N₃ > MeO >> CH₂=CH- against LCC6-WT (Pgp-) for the *meta*-substituted C2-benzoyl moiety, reflecting the taxoids's ability to bind microtubules. The potency order changes to MeO > N₃ > Cl > F >> CH₂=CH- against LCC6-MDR (Pgp+), which reflects their effect on MDR reversal activity or simply indicates the extent of their interaction with Pgp in the reverse order. The C-3' 2-methylprop-1-enyl and 2-methylpropyl groups are the best substituents for enhanced potency so far. However **12m-12p** are also very active. All these highly potent new taxoids are very good candidates for further preclinical studies.²⁹

Cytotoxicity of selected new generation taxoids **SB-T-1214**, **12g** and **13g** was examined against paclitaxel-resistant cancer cells with point mutations in tubulin to test their ability to deal with drug resistance other than MDR.²⁹ Two paclitaxel-resistant sublines 1A9PTX10 and 1A9PTX22 with point mutations in the class I β -tubulin have been reported.⁵⁷ As Table 8 shows, all three taxoids exhibit extremely potent activity, especially against drug-resistant cell lines 1A9PTX10 and 1A9PTX22, with two orders of magnitude higher potency than paclitaxel. These results clearly indicate that these second- and third-generation taxoids possess the capability to effectively circumvent the paclitaxel drug-resistance arising from point mutations in tubulins/microtubules besides MDR, which makes these new generation taxoids even more attractive.²⁹

Pancreatic cancer is refractory to conventional therapy and a major factor for such drug resistance is the expression of various multidrug resistance proteins.⁵⁸⁻⁶⁰ Our RT-PCR analysis showed that the CFPAC-1 and PANC-1 cell lines expressed the *mdr1*, *mrp1*, *mrp2* and *lrp* genes, responsible for multidrug resistance, while the MIA PaCa-2 and BxPC-3 cell lines expressed the *mrp1*, *mrp2* and *lrp* genes.⁶¹ Two taxoids, **SB-T-1214** and **12g** were evaluated against four pancreatic cancer cell lines, MIA PaCa-2, CFPAC-1, BxPC-3 and PANC-1. The results are shown in Table 9.²⁹

These taxoids exhibited excellent cytotoxicity against pancreatic cancer cell lines except for **12g** against PANC-1. Also, the third-generation taxoid, **12g**, which is more potent than **SB-T-1214** (>10 times) against the Pgp+ (i.e., *mdr1*) NCI/ADR cell line (see Table 7) shows a lower potency than **SB-T-1214** against the BxPC-3 and PANC-1 cell lines. This may indicate that **12g** cannot modulate a combination of multidrug-resistant proteins as efficiently as Pgp alone but it is still highly cytotoxic to PANC-1. **SB-T-1214** exhibits very high potency against these cell lines and did not show any appreciable cytotoxicity against primary pancreatic ductal cells up to 10 μ M concentration. A preliminary in vivo efficacy assay (20 mg/kg $\times$ 3, 60 mg/kg total dose in Tween 80/EtOH/PBS) against a pancreatic cancer CFPAC-1 xenograft in nude mice showed high efficacy with no trace of cancer cells by histopathological analysis after 8 weeks.²⁹

The antitumor activity of **SB-T-1214**, one of the leading candidates among the new generation taxoids studied in our laboratory, was assayed in vivo against a Pgp+ DLD-1 human colon tumor xenograft in SCID mice.²⁹ The taxoid was administered intravenously at three doses three times using a 3 day regimen (q3d $\times$ 3, on day 5, 8, and 11), starting from day 5 after DLD-1 subcutaneous tumor implantation. As Table 10 shows, the optimal efficacy was obtained at 60 mg/kg total dose (20mg/kg $\times$ 3), wherein complete regression of DLD-1 tumors was achieved in five of five mice (tumor growth delay >150 days).²⁹ A systemic toxicity profile showed that there was only 3-5% weight loss during the period of day 15 to day 20, and the drug was very well tolerated by animals.²⁹ This promising result warrants further preclinical evaluation of this taxoid.

The second-generation fluoro-taxoids, **21** and **22**, were evaluated for their cytotoxicity in vitro against various cell lines, as summarized in Table 11 and Table 12, respectively.³⁶ The IC₅₀ values were determined through 72 h exposure of the fluoro-taxoids to the cancer cells, following the procedure developed by Skehan et al.⁶² These fluoro-taxoids possess substantially higher potencies than those of paclitaxel and docetaxel against drug-sensitive cancer cell lines (except for a few cases) and their potency against multidrug-resistant cell lines is more impressive (two orders of magnitude more potent than paclitaxel in average). The potency of 3'-CF₂H-taxoids **21** against MCF7-S and LCC6-WT appears to be higher and more uniform with different substitution patterns as compared to that of 3'-CF₃-taxoids **22**, except for two cases (SB-T-12822-1: 0.19 nM, MCF7-S; SB-T-12824-1: 0.17 nM, MCF7-S). On the contrary, **22** exhibit more uniform potency against multidrug-resistant MCF7-R and LCC6-MDR cell lines than **21**. For **21**, cytotoxicity against these cell lines depends on the nature of *meta* substituents of the C-2-benzoate moiety, i.e., potency tends to increase in the order: F < MeO < Cl < N₃. In contrast, no clear trend is observed for **22** against these multidrug-resistant cell lines. SB-T-12842-4 appears to be the most potent compound with a R/S ratio of only 2.9-3.0 against two sets of human breast cancer cell lines.

The novel C-seco-fluorotaxoids, **28a** and **28b**, were evaluated against several drug-sensitive and drug-resistant ovarian cancer cell lines, and the results are summarized in Table 13. Drug-resistance in the A2780ADR cell line is based on MDR, while that in the A2780TC1 and A2780TC3 cell lines is caused by the overexpression of the class III β -tubulin subunit and other possible mutations. Thus, the activity of these two C-seco-fluorotaxoids is of particular interest. As Table 13 shows, **28a** is 39 times more potent than paclitaxel against the A2780TC3 cell line. The resistance factor for A2780TC3, i.e., IC₅₀ (A2780TC3)/IC₅₀ (A2780wt), is 10,470 for paclitaxel, but it is only 41 for **28a**. For comparison, IDN5390 exhibits 8.0 times higher potency than paclitaxel with the resistance factor of 129 against the same cell line.

The C-3'-substituents of C-seco-fluorotaxoids **28a** (2-methylpropyl) and **28b** (2-methylprop-1-enyl) also show interesting effects on potency, which is assumed to be related directly to their interaction with the class III β -tubulin. Overall, it has been shown that the

introduction of one fluorine to the C-2-benzoate moiety of C-seco-taxoid molecule substantially increases the potency against both paclitaxel-sensitive and paclitaxel-resistant human ovarian cancer cell lines.

The cytotoxicities of 3'-difluorovinyl-taxoids **33** were evaluated against several cancer cell lines. As Table 14 shows, these taxoids are exceedingly potent as compared to paclitaxel.⁴² The *meta* substitution of C-2-benzoate has a clear effect on the potency against drug-sensitive and drug-resistant MCF7 cell lines (entries 2-5 vs. entries 6-11). Difluorovinyl-taxoids with 2,10-modifications (entries 6-11) also possess impressive potency. SB-T-12853 appears particularly promising against gastrointestinal (GI) cancer cell lines.

The cytotoxicity of the novel macrocyclic taxoids was evaluated against several drug-sensitive and drug-resistant cell lines. As Table 15 shows, taxoid **43b** (SB-T-2054) was the most potent compound.²⁴ The results may suggest that **43b** is very closely mimicking paclitaxel's bioactive conformation. The results appear to indicate high sensitivity of the potency to the subtle difference in the position of the C-3'*N*-benzoyl group, the rigidity of the macrocyclic structure and the ring size. Also, there is a marked difference between the potency of the *E* isomer and the *Z* isomer of **34c**.

The activity of **43b** (SB-T-2054) was also evaluated in an in vitro tubulin polymerization assay using paclitaxel as the standard for comparison. As Figure 6 shows, **43b** induced tubulin polymerization in the absence of GTP in a manner similar to paclitaxel and the microtubules formed with both were stable against Ca²⁺-induced depolymerization.²⁴ This result also supports our observation that **43b** is closely mimicking paclitaxel's bioactive conformation (see Figure 7).²⁴

Conclusion

New-generation taxoids with systematic and strategic modifications were designed, synthesized and examined for their potency and efficacy in vitro and in vivo. As a result, it has been shown that a number of these taxoids are exceptionally potent. Some of these taxoids exhibited very low resistance factors i.e., virtually overcoming multi-drug resistance completely. Thus, these taxoids have been termed "third-generation" taxoids. Novel fluorine-containing taxoids were also investigated. For example, 3'-difluorovinyl-taxoids have been designed to block the metabolic hydroxylation by cytochrome P-450 enzymes and these were found to exhibit exceptionally high potency against several cancer cell lines. Novel C-14-C-3' BzN-linked macrocyclic taxoids were designed to mimic the "REDOR-Taxol" structure. One of the macrocyclic taxoids was found to possess virtually the same potency as that of paclitaxel, which suggests that its structure almost perfectly mimics the bioactive conformation of paclitaxel. The encouraging profiles of some of these new generation taxoids make them highly promising candidates for further preclinical studies.

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Figure 1.

Figure 2.
Primary sites of hydroxylation on the second-generation taxoids by the P450 family of enzymes⁴¹

Figure 3.
Designed novel C-14-C-3'BzN-linked macrocyclic taxoids

Figure 4.
Overlays of REDOR-Taxol (green) with **34a** (cyan), **34b** (red) and **34c** (pink).

Figure 5.
X-ray crystal structure of **43b** (SB-T-2054)

Figure 6. Tubulin polymerization with **43b** and paclitaxel: microtubule protein 1mg/mL, 37 °C, GTP 1 mM or Drug 10 μ M

Figure 7.
Overlays of REDOR-Taxol (green) with **43b** (SB-T-2054) (purple).

Scheme 1

Reagents and conditions: (i) (a) TESCl, imidazole, (b) LiHMDS, AcCl, 95% in two steps; (ii) LiHMDS, THF, 95%; (iii) $\text{N}_2\text{H}_4 \cdot \text{H}_2\text{O}$, EtOH, 85%

Scheme 2

Reagents and conditions: (i) (a) RCl, TEA, DMAP, CH₂Cl₂ or (b) ROH, DIC, DMAP, CH₂Cl₂ or (c) RCl, LiHMDS, THF, -20 °C (75-98%); (ii) HF/pyridine, pyridine/MeCN (80-97%).

Scheme 3

Reagents and conditions: (i) TESCl, imidazole, DMF, RT, 96%; (ii) sodium bis(2-methoxyethoxy)aluminumhydride, THF, -10 °C, 97%; (iii) DIC, DMAP, CH₂Cl₂, 85-90%; (iv) HF/pyridine, pyridine/MeCN; (v) TESCl, imidazole, DMF, RT, 2 h 71-95% for two steps; (vi) LiHMDS, R²COCl, THF, 67-98%.

Scheme 4

Reagents and conditions: (i) **3a-f** (1.2~1.5 eq), LiHMDS, THF, -40 °C, 30 min; (ii) HF/pyridine, pyridine/MeCN, 0 °C – RT, 18 h, 65-95% for two steps; (iii) $\text{H}_2/\text{Pd-C}$, EtOAc/MeOH, RT, 24 h, 70-89%.

Scheme 5

Reagents and conditions: (i) LiHMDS, THF, $-40\text{ }^{\circ}\text{C}$, 30 min; (ii) HF/pyridine, pyridine/MeCN, $0\text{ }^{\circ}\text{C}$ – RT, 18 h (61-86% for two steps); (iii) H_2 , Pd/C, EtOAc, RT, 24 h (95-98%).

R = MeCO, EtCO, N(Me)₂CO, MeOCO, TES; **X** = MeO, F, Cl, N₃; **Rf** = CF₂H, CF₃

Scheme 6

Scheme 7

Reagents and conditions: i) $\text{Cu}(\text{OAc})_2$, MeOH, 77-86 %; ii) L-selectride , THF, -78°C 50-70%, iii) methyl imidazole, TESC1, DMF, 0°C , 50-80 %; iv) LiHMDS, THF, -40°C , 70-80%; v) HF/pyridine, CH_3CN /pyridine, 0°C - RT, 52%-92%.

Scheme 8

Reagents and conditions: i) CBr₂F₂, HMPT, Zn, THF, 84%; ii) CAN, H₂O/CH₃CN, -15 °C, 92%; iii) Boc₂O, Et₃N, DMAP, CH₂Cl₂, 96%; iv) LiHMDS, THF, -40 °C, v) HF/Py, Py/CH₃CN, overnight, 0 °C-RT, 57-91%.

35a: $R^1 = \text{Ph}$, $R^2 = \text{TES}$

3b: $R^1 = \text{Me}_2\text{C}=\text{CH}$, $R^2 = \text{TIPS}$

36a: $X = \text{CH}_2$

36b: $X = \text{none}$

36c: $X = \text{OCH}_2$

37a: $R^1 = \text{Ph}$, $R^2 = \text{TES}$, $X = \text{CH}_2$ (91%)

37b: $R^1 = \text{Ph}$, $R^2 = \text{TES}$, $X = \text{bond}$ (90%)

37c: $R^1 = \text{Ph}$, $R^2 = \text{TES}$, $X = \text{OCH}_2$ (99%)

37d: $R^1 = \text{Me}_2\text{C}=\text{CH}$, $R^2 = \text{TIPS}$, $X = \text{CH}_2$ (80%)

Scheme 9

Reagents and conditions: (i) acid chloride (2.0 equiv.), Et_3N (4 equiv.), CH_2Cl_2 , DMAP, overnight.

Scheme 10

Reagents and conditions: (i) (a) Ac_2O (10 eq), $\text{CeCl}_3 \cdot 7\text{H}_2\text{O}$ (0.1 eq), THF, r.t., 2 h, (b) TESCl (3.0 eq), Imidazol (4.0 eq), DMF, r.t., 5 h (83% in 2 steps), (ii) Allyl iodide (1.1 eq), NaHMDS (1.1 eq), DMF, -40°C , 1 h (82%), (iii) **37a-d** (3.0 eq), LiHMDS (1.5 eq), THF, $-30^\circ\text{C} \sim 0^\circ\text{C}$, 2.5 h.

Scheme 11

Reagents and conditions: (i) (a) $\text{Cl}_2\text{Ru}(=\text{CHPh})(\text{PCy}_3)_2$ (0.2 eq), CH_2Cl_2 , overnight; (ii) HF/Py , Py , CH_3CN , r.t., overnight

Scheme 12

Reagents and conditions: (i) (a) $\text{Cl}_2\text{Ru}(\text{=CHPh})(\text{PCy}_3)_2$ (0.2 eq), CH_2Cl_2 , overnight, flash chromatography; (ii) HF/Py , Py , CH_3CN , r.t., overnight.

Scheme 13

Reagents and conditions: (i) (a) $\text{Cl}_2\text{Ru}(=\text{CHPh})(\text{PCy}_3)_2$ (0.25 eq $\times$ 3), CH_2Cl_2 , reflux, 5d; (ii) HF/Py , Py , CH_3CN , RT, overnight

Table 1Synthesis of Taxoids **6**

taxoid	R
6a	Bz
6b	2-MeO(C ₆ H ₄)CO
6c	3-MeO(C ₆ H ₄)CO
6d	4-MeO(C ₆ H ₄)CO
6e	3,4-(MeO) ₂ (C ₆ H ₃)CO
6f	1-naphthoyl
6g	2-naphthoyl
6h	Cbz
6i	2-MeO(C ₆ H ₄)CH ₂ CO
6j	3-MeO(C ₆ H ₄)CH ₂ CO
6k	4-MeO(C ₆ H ₄)CH ₂ CO
6l	(C ₆ H ₅)(CH ₂) ₂ CO
6m	2-MeO(C ₆ H ₄)(CH ₂) ₂ CO
6n	3-MeO(C ₆ H ₄)(CH ₂) ₂ CO
6o	4-MeO(C ₆ H ₄)(CH ₂) ₂ CO

Table 22,10-Modified Baccatins **11**

baccatin	R ¹	R ²
11a	Me	MeCO
11b	MeO	MeCO
11c	F	EtCO
11d	Cl	EtCO
11e	N ₃	EtCO
11f	CH ₂ =CH-	EtCO
11g	MeO	EtCO
11h	MeO	<i>c</i> -PrCO
11i	MeO	MeOCO
11j	MeO	PhCH ₂ OCO
11k	MeO	2-MeO(C ₆ H ₄)CO
11l	MeO	4-MeO(C ₆ H ₄)CH ₂ CO

Table 3
Second- and Third-generation Taxoids **12** and **13**.

taxoid	R ¹	R ²	R ³
12a	Me	MeCO	Me ₂ C=CH-
12b	MeO	MeCO	Me ₂ C=CH-
12c	F	EtCO	Me ₂ C=CH-
12d	Cl	EtCO	Me ₂ C=CH-
12e	N ₃	EtCO	Me ₂ C=CH-
12f	CH ₂ =CH-	EtCO	Me ₂ C=CH-
12g	MeO	EtCO	Me ₂ C=CH-
12h	MeO	<i>c</i> -PrCO	Me ₂ C=CH-
12i	MeO	MeOCO	Me ₂ C=CH-
12j	MeO	PhCH ₂ OCO	Me ₂ C=CH-
12k	MeO	2-MeO(C ₆ H ₄)CO	Me ₂ C=CH-
12l	MeO	4-MeO(C ₆ H ₄)CH ₂ CO	Me ₂ C=CH-
12m	MeO	EtCO	CH ₂ =CHCH ₂ -
12n	MeO	EtCO	(<i>E</i>)-CH ₃ CH=CH-
12o	MeO	EtCO	CH ₂ =CH(CH ₂) ₂ -
12p	MeO	EtCO	(<i>S</i>)-2,2-Me ₂ - <i>c</i> -Pr-
13c	F	EtCO	Me ₂ CHCH ₂ -
13d	Cl	EtCO	Me ₂ CHCH ₂ -
13e	N ₃	EtCO	Me ₂ CHCH ₂ -
13g	MeO	EtCO	Me ₂ CHCH ₂ -

Table 4
C-3' *N*-Modified Second-generation Taxoids **15** and **16**

taxoid	R ¹	R ⁴
15a	H	cyclobutyl
15b	H	cyclopentyl
15c	H	cyclohexyl
15d	H	cyclopent-1-enyl
15e	H	cyclohex-1-enyl
15f	H	cyclopentyloxy
15g	H	cyclohexyloxy
15h	H	cyclopropyl
15i	H	cyclobutyl
15j	H	cyclopentyl
15k	H	cyclohexyl
15l	H	cyclohexyloxy
16b	H	cyclopentyl
16c	H	cyclohexyl
16f	H	cyclopentyloxy
16g	H	cyclohexyloxy
16h	MeO	cyclopropyl
16k	MeO	cyclohexyl
16m	MeO	cyclopentyloxy

Table 5Cytotoxicity of Second-generation Taxoids with Modifications at C-10 (IC_{50} nM)^a

taxoid	R
paclitaxel	--
6a	Bz

R

6b

taxoid

R

6c

taxoid

R

6d

taxoid

R

6e

6f

taxoid

R

6g

6h

Cbz

taxoid

R

6i

taxoid

R

6j

6k

6l

taxoid

R

6m

6n

6o

^aConcentration of compound that inhibits 50% (IC₅₀, nM) of the growth of human tumor cell line after a 72 h drug exposure.

^bLCC6-WT: human breast carcinoma cell line (Pgp-).

^cLCC6-MDR: *mdr1* transduced cell line (Pgp+).

^dResistance factor = (IC₅₀ for drug resistant cell line, R)/(IC₅₀ for drug-sensitive cell line, S).

Table 6
Cytotoxicity of Second-generation Taxoids with C-3'N Modifications (IC₅₀ nM)^a

taxoid	R ¹	R ²	LCC6-WT ^b	LCC6-MDR ^c	R/S ^d	MCF7 ^e	NCI/ADR ^f	R/S ^d
paclitaxel	--	--	3.1	346	112	1.7	300	176
SB-T-1213	<i>t</i> -Boc	2-Me-prop-1-enyl	--	--	--	0.18	4.0	22
SB-T-1103	<i>t</i> -Boc	2-Me-propyl	--	--	--	0.35	5.1	15
15a	cyclobutyl	2-Me-prop-1-enyl	1.3	34	26	1.2	41	34
15b	cyclopentyl	2-Me-prop-1-enyl	1.1	19	17	1.1	22	20
16b	cyclopentyl	2-Me-propyl	6.3	64	10	2.3	29	12
15c	cyclohexyl	2-Me-prop-1-enyl	1.3	13	10	1.0	18	18
16c	cyclohexyl	2-Me-propyl	5.8	32	5.5	1.5	19	13
15d	cyclopent-1-enyl	2-Me-prop-1-enyl	1.2	14	12	0.4	6.0	15
15e	cyclohex-1-enyl	2-Me-prop-1-enyl	1.2	11	9.2	0.3	3.8	13
15f	cyclopentyloxy	2-Me-prop-1-enyl	0.94	12	13	1.4	8.6	6.1
16f	cyclopentyloxy	2-Me-propyl	2.6	14	5.4	1.3	8.8	6.8
15g	cyclohexyloxy	2-Me-prop-1-enyl	1.2	15	13	1.4	8.9	6.4
16g	cyclohexyloxy	2-Me-propyl	4.8	18	3.8	1.4	14	10

^a See the footnotes of Table 5.

^b See the footnote of Table 5.

^c See the footnote of Table 1.

^d See the footnote of Table 5.

^e MCF7: human breast carcinoma cell line.

$f_{\text{NCI/ADR}}$: multidrug-resistant human ovarian carcinoma cell line.

Table 7
Cytotoxicity of Second-generation Taxoids with C-2-*meta* Modifications (IC₅₀ nM)^a

taxoid	R ¹	R ²	R ³	R ⁴	LCC6-WT ^b	LCC6-MDR ^c	R/S ^d	MCF7 ^e	NCI/ADR ^f	R/S ^d
paclitaxel	Ph	Ph	MeCO	H	3.1	346	112	1.7	300	176
docetaxel	<i>t</i> -BuO	Ph	H	H	1.0	120	120	1.0	235	235
SB-T-1213	<i>t</i> -BuO	Me ₂ C=CH-	EtCO	H	--	--	--	0.18	4.0	22
SB-T-1103	<i>t</i> -BuO	Me ₂ CHCH ₂ -	EtCO	H	--	--	--	0.35	5.1	21
SB-T-1214	<i>t</i> -BuO	Me ₂ C=CH-	<i>c</i> -PrCO	H	--	--	--	0.20	3.9	20
12a	<i>t</i> -BuO	Me ₂ C=CH-	MeCO	Me	1.5	5.8	3.9	0.8	5.0	6.3
12b	<i>t</i> -BuO	Me ₂ C=CH-	MeCO	MeO	0.6	2.7	4.5	0.8	2.3	2.9
12c	<i>t</i> -BuO	Me ₂ C=CH-	EtCO	F	0.5	2.1	4.2	--	--	--
12d	<i>t</i> -BuO	Me ₂ C=CH-	EtCO	Cl	0.8	1.3	1.6	--	--	--
12e	<i>t</i> -BuO	Me ₂ C=CH-	EtCO	N ₃	0.9	1.2	1.3	0.9	1.1	1.2
12f	<i>t</i> -BuO	Me ₂ C=CH-	EtCO	CH ₂ =CH-	2.9	7.1	2.4	--	--	--
12g	<i>t</i> -BuO	Me ₂ C=CH-	EtCO	MeO	1.0	0.9	0.90	0.36	0.33	0.92
12h	<i>t</i> -BuO	Me ₂ C=CH-	<i>c</i> -PrCO	MeO	1.0	2.9	2.9	--	--	--
12i	<i>t</i> -BuO	Me ₂ C=CH-	MeOCO	MeO	0.6	1.6	2.7	0.4	1.4	3.5
12j	<i>t</i> -BuO	Me ₂ C=CH-	PhCH ₂ OCO	MeO	1.2	1.8	1.5	0.2	1.5	7.5
12k	<i>t</i> -BuO	Me ₂ C=CH-	2-MeO(C ₆ H ₄)CO	MeO	0.4	0.9	2.3	1.1	3.3	3.0
12l	<i>t</i> -BuO	Me ₂ C=CH-	4-MeO(C ₆ H ₄)CH ₂ CO	MeO	0.4	0.4	1.0	0.6	1.8	3.0
12m	<i>t</i> -BuO	CH ₂ =CHCH ₂ -	EtCO	MeO	1.2	8.4	7.0	0.8	8.7	10.9

taxoid	R ¹	R ²	R ³	R ⁴	LCC6-WT ^b	LCC6-MDR ^c	R/S ^d	MCF7 ^e	NCI/ADR ^f	R/S ^d
12n	<i>t</i> -BuO	(<i>E</i>)-CH ₃ CH=CH-	EtCO	MeO	1.2	4.1	3.4	--	--	--
12o	<i>t</i> -BuO	CH ₂ =CH(CH ₂) ₂ -	EtCO	MeO	0.9	5.4	6.0	2.0	7.7	3.9
12p	<i>t</i> -BuO	(<i>S</i>)-2,2-Me ₂ - <i>c</i> -Pr-	EtCO	MeO	0.48	1.1	2.3	0.6	1.5	2.5
13c	<i>t</i> -BuO	Me ₂ CHCH ₂ -	EtCO	F	0.4	2.4	6.0	--	--	--
13d	<i>t</i> -BuO	Me ₂ CHCH ₂ -	EtCO	Cl	0.8	2.9	3.6	--	--	--
13e	<i>t</i> -BuO	Me ₂ CHCH ₂ -	EtCO	N ₃	1.1	2.4	2.2	1.0	2.1	2.1
13g	<i>t</i> -BuO	Me ₂ CHCH ₂ -	EtCO	MeO	0.9	0.8	0.89	0.36	0.43	1.19
15h	<i>c</i> -Pr	Me ₂ C=CH-	EtCO	MeO	1.1	17.2	16	0.5	7.8	15.6
15i	<i>c</i> -Bu	Me ₂ C=CH-	EtCO	MeO	1.6	15	9.4	0.8	8.8	11
15j	<i>c</i> -Pentyl	Me ₂ C=CH-	EtCO	MeO	1.1	11	10	0.3	9.5	32
15k	<i>c</i> -Hexyl	Me ₂ C=CH-	EtCO	MeO	6.9	42	6.1	1.8	17.5	9.7
15l	<i>c</i> -Hex-O	Me ₂ C=CH-	EtCO	MeO	1.23	14.8	12.0	1.44	14	9.7
16h	<i>c</i> -Pr	Me ₂ CHCH ₂ -	EtCO	MeO	0.6	13	22	0.4	11.8	30
16k	<i>c</i> -Hexyl	Me ₂ CHCH ₂ -	EtCO	MeO	1.0	12	12	0.7	6.5	9.3
16m	<i>c</i> -Pent-O	Me ₂ CHCH ₂ -	EtCO	MeO	0.76	2.6	3.4	0.17	1.18	6.9

^a See the footnote of Table 5.

^b See the footnote of Table 5.

^d See the footnote of Table 5.

^e See the footnote of Table 6.

^f See the footnote of Table 6.

Table 8
Cytotoxicity of New-generation Taxoids against the 1A9PTX10 and 1A9PTX22 Cell Lines (IC₅₀, nM)^a

taxoid
	A-2780	1A9PTX10	R/S ^b	1A9PTX22
paclitaxel	1.38±0.05	532.95±3.18	386	160.70±14.70
SB-T-1214	0.44±0.04	9.00±0.77	20.4	3.94±0.03
12g	0.76±0.01	3.65±0.21	4.8	3.88±0.54
13g	0.25±0.01	4.91±0.53	19.6	2.10±0.13

^a See the footnote of Table 5.

^b See the footnote of Table 5.

Table 9Cytotoxicity of **SB-T-1214** and **12g** against Pancreatic Cancer Cell Lines (IC₅₀ nM)^a

taxoid	MIA PaCa-2	CFPAC-1	BxPC-3	PANC-1
SB-T-1214	0.92	0.83	1.04	3.68
12g	0.68	0.89	3.03	22.6

^aSee the footnote of Table 5.

Table 10Antitumor Effect of **SB-T-1214** Delivered i.v. to SCID Mice Bearing a Pgp+ Human Colon Tumor Xenograft, DLD-1

treatment ^a (i.v.)	total dose (mg/kg)	growth delay (days)	toxicity ^c	cured mice / group ^d
control	0	---	0	0 / 10
vehicle	0	4	0	0 / 5
paclitaxel	60	8	0	0 / 5
SB-T-1214	30	37	0	0 / 5
SB-T-1214	60	>150	0	5 / 5
SB-T-1214	120	>150	2	3 / 5

^aTreatment given iv to SCID mice on day 5 after DLD-1 human colon tumor implant, and continued as noted, with all drugs formulated in Tween/EtOH^bBased on comparison of each group vs. control using the Cox-Mantel test.^cNumber of animals who either died or lost greater than 20% body weight.^dSCID mice with no palpable tumors on day 167, the end of experiment.

Table 11
In vitro Cytotoxicity (IC₅₀ nM)^a of C-3'-CF₂H-taxoid (21)

taxoid	R	X	MCF7-S ^b (breast)	MCF7-R ^c (breast)	R/S ^d	LCC6-WT ^b (breast)	LCC6-MDR ^e (breast)	R/S ^d	H460 ^f (lung)	HT-29 ^g (colon)
paclitaxel			1.7	300	176	3.1	346	112	4.9	3.6
docetaxel			1.0	215	215	...	...	...	...	1.0
SB-T-12841-1	Ac	MeO	0.34	4.16	12	0.26	5.57	21	0.38	0.52
SB-T-12841-2	Ac	F	0.44	5.33	13	0.52	10.0	19	0.20	0.35
SB-T-12841-3	Ac	Cl	0.40	6.48	16	0.31	5.80	19	0.49	1.94
SB-T-12841-1	Ac	N ₃	0.32	1.68	5.3	0.22	1.57	7.1	0.48	0.57
SB-T-12842-1	Et-CO	MeO	1.14	4.05	3.5	0.69	4.92	7.1	0.40	0.59
SB-T-12842-2	Et-CO	F	0.53	7.24	14	0.88	4.63	3.5	0.41	0.86
SB-T-12842-3	Et-CO	Cl	0.44	5.20	12	0.52	4.71	9.1	0.30	0.43
SB-T-12842-4	Et-CO	N ₃	0.32	0.96	3.0	0.39	1.15	2.9	0.27	0.37
SB-T-12843-1	Me ₂ N-CO	MeO	0.45	4.51	10	0.69	7.06	10	0.40	0.43
SB-T-12843-2	Me ₂ N-CO	F	0.52	8.13	16	0.69	10.6	15	0.20	0.35
SB-T-12843-3	Me ₂ N-CO	Cl	0.31	2.96	9.5	0.21	3.87	18	0.36	0.58
SB-T-12843-4	Me ₂ N-CO	N ₃	0.37	1.44	3.9	0.29	1.69	5.8	0.52	0.40
SB-T-12844-1	MeO-CO	MeO	0.81	6.59	8.1	1.03	10.2	9.9	0.30	0.44
SB-T-12844-2	MeO-CO	F	0.59	11.38	19	0.86	12.6	15	0.30	0.43
SB-T-12844-3	MeO-CO	Cl	0.26	2.08	8.0	0.13	1.82	14	0.25	0.29
SB-T-12844-4	MeO-CO	N ₃	1.69	2.56	1.5	0.26	2.06	7.9	0.23	0.36

^{a-e} See footnote ^a of Table 5.
^f Human non-small cell lung carcinoma.
^g Human Caucasian colon adenocarcinoma.

Table 12
In vitro Cytotoxicity (IC₅₀ nM)^a of C-3'-CF₃-taxoids (22)

taxoid	R	X	MCF7-S ^b (breast)	MCF7-R ^c (breast)	R/S ^d	LCC6-WT ^b (breast)	LCC6-MDR ^e (breast)	R/S ^d	H460 ^f (lung)	HT-29 ^g (colon)
paclitaxel			1.7	300	176	3.1	346	112	4.9	3.6
docetaxel			1.0	215	215	...	...	...	...	1.0
SB-T-12821-1	Ac	MeO	0.32	8.8	28	0.33	3.99	12	0.38	0.69
SB-T-12821-2	Ac	F	0.45	5.58	13	0.38	5.93	16	0.49	1.11
SB-T-12821-3	Ac	Cl	0.40	5.04	13	0.22	4.96	23	0.5	0.85
SB-T-12821-4	Ac	N ₃	0.47	3.85	8.2	1.18	4.00	3.4	0.20	0.50
SB-T-12822-1	Et-CO	MeO	0.19	2.16	11	0.45	4.24	9	0.41	0.54
SB-T-12822-2	Et-CO	F	0.68	3.78	5.6	0.82	4.27	5.2	0.59	1.15
SB-T-12822-3	Et-CO	Cl	0.34	3.28	9.6	0.39	2.54	6.5	0.63	1.11
SB-T-12822-4	Et-CO	N ₃	0.38	1.61	4.2	1.09	2.56	2.3	0.20	0.40
SB-T-12823-1	Me ₂ NCO	MeO	0.57	1.84	3.2	0.28	4.48	16	0.35	0.68
SB-T-12823-2	Me ₂ NCO	F	0.32	2.64	8.3	0.32	5.57	17	0.5	0.76
SB-T-12823-3	Me ₂ NCO	Cl	0.12	1.02	8.5	0.27	2.55	9.4	0.42	0.45
SB-T-12823-4	Me ₂ NCO	N ₃	0.47	2.61	5.6	1.27	3.52	2.8	0.30	0.50
SB-T-12824-1	MeOCO	MeO	0.17	2.88	17	0.27	3.99	15	0.38	0.53
SB-T-12824-2	MeOCO	F	0.31	4.88	16	0.39	5.81	15	0.61	0.85
SB-T-12824-3	MeOCO	Cl	0.65	4.72	7.3	0.29	5.08	18	0.43	0.68
SB-T-12821-1	MeOCO	N ₃	0.47	2.92	6.2	1.09	4.00	3.7	0.20	0.40

^{a-g}See footnotes of Table 11.

Table 13
In vitro Cytotoxicity (IC₅₀ nM)^a of C-seco-fluorotaxoids

C-seco-taxoid	A2780wt ^b	A2780CIS ^c	A2780TOP ^d	A2780ADR ^e	A2780TC1 ^f	A2780TC3 ^g
paclitaxel	1.7	2.2	7.2	1,239	10,027	17,800
IDN 5390	17.4	16.8	27.5	2,617	2,060	2,237
28a (SB-CST-10104)	11.1	11.8	12.8	3,726	1,497	460
28b (SB-CST-10204)	6.1	4.9	6.9	2,218	4,454	745

^a See footnote of Table 5.
^b Human ovarian carcinoma wild type.
^c Cisplatin-resistant A2780.
^d Topotecan-resistant A2780.
^e Adriamycin-resistant A2780.
^{f,g} Clones derived from chronic exposition of A2780 to paclitaxel and cyclosporine.

Table 14

In vitro Cytotoxicity (IC₅₀ nM)^a of 3'-Difluorovinyl-taxoids 33

entry	taxoid	R	X	MCF7-S ^b (breast)	MCF7-R ^c (breast)	R/S	HT-29 ^d (colon)	PANC-1 ^e (pancreatic)
1	paclitaxel			1.2	300	250	3.6	25.7
2	SB-T-12851	Ac	H	0.099	0.95	9.6	0.41	1.19
3	SB-T-12852	c-Pr-CO	H	0.12	6.0	50	0.85	5.85
4	SB-T-12853	Et-CO	H	0.12	1.2	10	0.34	0.65
5	SB-T-12854	Me ₂ N-CO	H	0.13	4.3	33	0.46	1.58
6	SB-T-12852-1	c-Pr-CO	MeO	0.092	0.48	5.2	...	...
7	SB-T-12853-1	Et-CO	MeO	0.34	0.57	1.7	...	...
8	SB-T-12855-1	MeO-CO	MeO	0.078	0.50	6.4	...	...
9	SB-T-12851-3	Ac	N ₃	0.092	0.34	3.7	...	...
10	SB-T-12852-3	c-Pr-CO	N ₃	0.092	0.45	4.9	...	...
11	SB-T-12855-3	MeO-CO	N ₃	0.078	0.40	5.3	...	...

^{a-d} See footnotes of Table 11.

^e Human pancreatic carcinoma.

Table 15
Cytotoxicity of C-14-C-3'BzN-linked Macrocyclic Taxoids (IC₅₀ nM)^a

taxoids	MCF-7 ^b	NCI/ADR ^c	LCC6-WT ^d	LCC6-MDR ^e	A2780wt ^f	HT-29 ^g
paclitaxel	1.85	395	2.45	110	36.1	7.28
34a (SB-T-2053)	12.3	592	12.2	300	114	29.2
43b (SB-T-2054)	3.49	183	2.09	129	31.0	17.0
34c-E	1.650	10,010	2,067	2,285	1,475	523
34c-Z	196	1,135	348	1,924	198	62.0
34d	398	1,000	130	618	---	---

^a See footnote of Table 5.
^{b, c} See footnote of Table 6.
^{d, e} See footnote of Table 5.
^f See footnote of Table 13.
^g See footnote of Table 11.