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

Iwao Ojima* and Manisha Das

Department of Chemistry and Institute of Chemical Biology & Drug Discovery, State University of New York at Stony Brook, Stony Brook, New York 11794-3400

Received October 15, 2008

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 United States.[1,2] Paclitaxel and docetaxel are among the most widely used chemotherapeutic drugs especially against some types of cancer such as 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 1−2 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 (MeO, N_3, 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 such as 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 8-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 design[19] 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 Georg[25] 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 of **12g** on Pd/C (Scheme 4).

Synthesis of C-3′N-Modified Taxoids

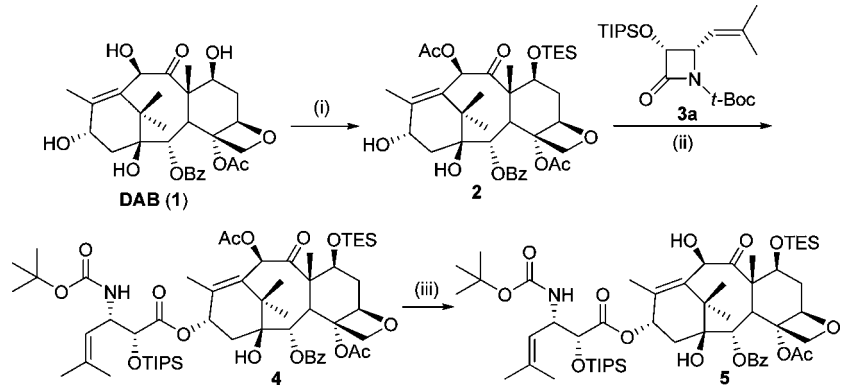
C-3′N-Modified taxoids **15** and **16** were synthesized using enantiopure β-lactams bearing various *N*-acyl or *N*-carbalkoxy groups (Scheme 5, Table 4).[29] These β-lactams were prepared by reacting NH-free 3-TBSO- or 3-TIPSO-β-lactam with acid chlorides or chloroformates. The resulting β-lactams **14a**−**h** were coupled with baccatin **11b** followed by deprotection of silyl groups to afford

‡ Dedicated to Dr. David G. I. Kingston of Virginia Polytechnic Institute and State University for his pioneering work on bioactive natural products.

* To whom correspondence should be addressed. Tel: +631-632-1339. Fax: +631-632-7942. E-mail: iojima@notes.cc.sunysb.edu.

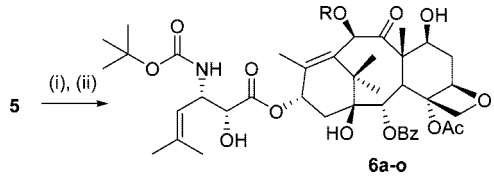
10.1021/np8006556 CCC: $40.75
Published on Web 02/24/2009

Scheme 1[a]



[a] Reagents and conditions: (i) (a) TESCl, imidazole, (b) LiHMDS, AcCl, 95% in two steps; (ii) LiHMDS, THF, 95%; (iii) $N_2H_4 \cdot H_2O$, EtOH, 85%.

Scheme 2[a]



[a] Reagents and conditions: (i) (a) RCl, TEA, DMAP, CH_2Cl_2 or (b) ROH, DIC, DMAP, CH_2Cl_2 or (c) RCl, LiHMDS, THF, −20 °C (75−98%); (ii) HF/pyridine, pyridine/MeCN (80−97%).

Table 1. Synthesis of Taxoids **6**

taxoid	R
6a	Bz
6b	2-MeO(C_6H_4)CO
6c	3-MeO(C_6H_4)CO
6d	4-MeO(C_6H_4)CO
6e	3,4-$(MeO)_2$(C_6H_3)CO
6f	1-naphthoyl
6g	2-naphthoyl
6h	Cbz
6i	2-MeO(C_6H_4)CH_2CO
6j	3-MeO(C_6H_4)CH_2CO
6k	4-MeO(C_6H_4)CH_2CO
6l	(C_6H_5)$(CH_2)_2$CO
6m	2-MeO(C_6H_4)$(CH_2)_2$CO
6n	3-MeO(C_6H_4)$(CH_2)_2$CO
6o	4-MeO(C_6H_4)$(CH_2)_2$CO

Table 2. 2,10-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	*c*-PrCO
11i	MeO	MeOCO
11j	MeO	$PhCH_2OCO$
11k	MeO	2-MeO(C_6H_4)CO
11l	MeO	4-MeO(C_6H_4)CH_2CO

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′-CF_2H− and C-3′-CF_3− groups, respectively, were synthesized from β-lactams **17** (R_f = CF_2H or CF_3) and baccatins **18** by means of the β-lactam synthon method,[35] as shown in Scheme 6.[36]

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

taxoid	R^1	R^2	R^3
12a	Me	MeCO	Me_2C═CH−
12b	MeO	MeCO	Me_2C═CH−
12c	F	EtCO	Me_2C═CH−
12d	Cl	EtCO	Me_2C═CH−
12e	N_3	EtCO	Me_2C═CH−
12f	CH_2═CH−	EtCO	Me_2C═CH−
12g	MeO	EtCO	Me_2C═CH−
12h	MeO	*c*-PrCO	Me_2C═CH−
12i	MeO	MeOCO	Me_2C═CH−
12j	MeO	$PhCH_2OCO$	Me_2C═CH−
12k	MeO	2-MeO(C_6H_4)CO	Me_2C═CH−
12l	MeO	4-MeO(C_6H_4)CH_2CO	Me_2C═CH−
12m	MeO	EtCO	CH_2═$CHCH_2$−
12n	MeO	EtCO	(*E*)-CH_3CH═CH−
12o	MeO	EtCO	CH_2═$CH(CH_2)_2$−
12p	MeO	EtCO	(*S*)-2,2-Me_2-*c*-Pr−
13c	F	EtCO	Me_2CHCH_2−
13d	Cl	EtCO	Me_2CHCH_2−
13e	N_3	EtCO	Me_2CHCH_2−
13g	MeO	EtCO	Me_2CHCH_2−

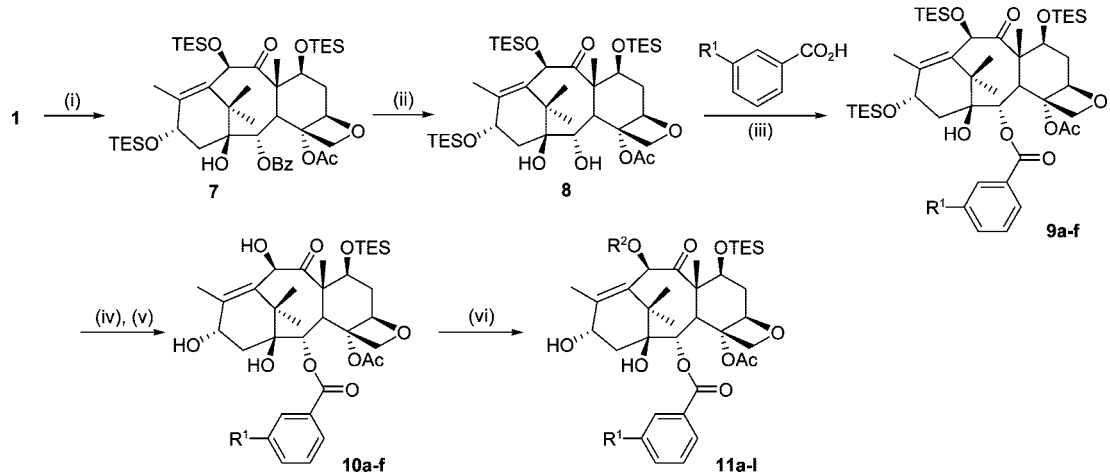
Table 4. C-3′N-Modified Second-Generation Taxoids **15** and **16**

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

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

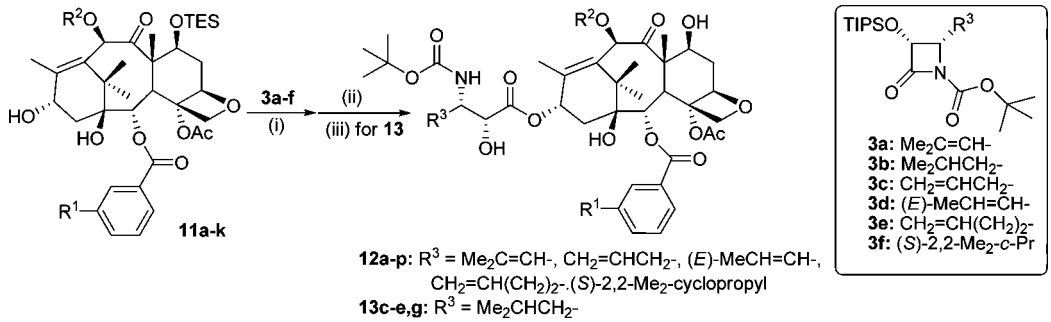
Recently, a C-seco-taxoid, IDN5390 (Figure 1), was reported to exhibit several times better potency than paclitaxel against drug-resistant ovarian cancer cell lines overexpressing the class III tubulin isotype.[16] As a part of our SAR study on IDN5390,

Scheme 3[a]



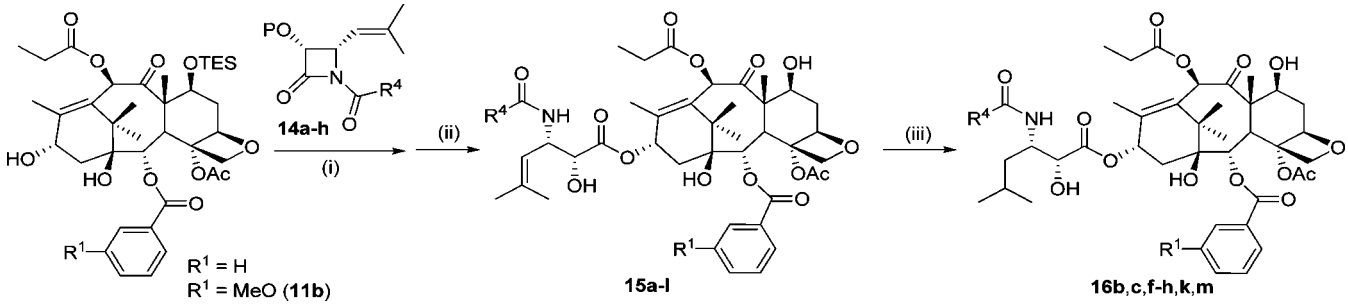
[a] Reagents and conditions: (i) TESCl, imidazole, DMF, RT, 96%; (ii) sodium bis(2-methoxyethoxy)aluminum hydride, THF, −10 °C, 97%; (iii) DIC, DMAP, CH_2Cl_2, 85−90%; (iv) HF/pyridine, pyridine/MeCN; (v) TESCl, imidazole, DMF, RT, 2 h 71−95% for two steps; (vi) LiHMDS, R^2COCl, THF, 67−98%.

Scheme 4[a]



[a] Reagents and conditions: (i) **3a**−**f** (1.2−1.5 equiv), LiHMDS, THF, −40 °C, 30 min; (ii) HF/pyridine, pyridine/MeCN, 0 °C − RT, 18 h, 65−95% for two steps; (iii) H_2/Pd−C, EtOAc/MeOH, RT, 24 h, 70−89%.

Scheme 5[a]



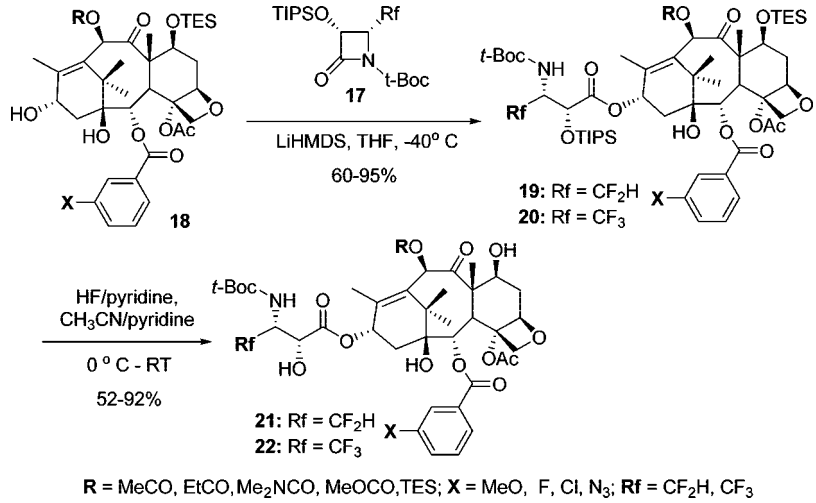
[a] Reagents and conditions: (i) LiHMDS, THF, −40 °C, 30 min; (ii) HF/pyridine, pyridine/MeCN, 0 °C to RT, 18 h (61−86% for two steps); (iii) H_2, Pd/C, EtOAc, RT, 24 h (95−98%).

we investigated two fluorine-containing analogues, 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: 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).

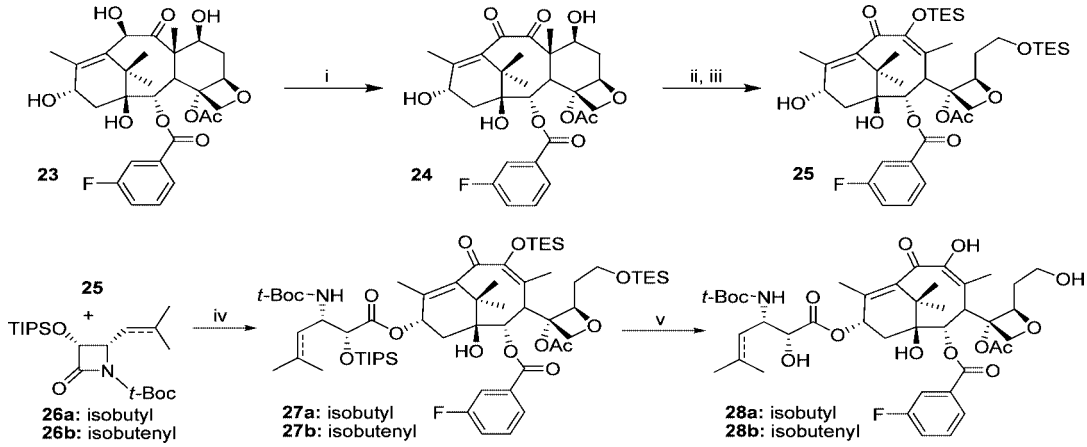
Synthesis of C-3′-Difluorovinyl-taxoids

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 *tert*-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

Scheme 6



Scheme 7[a]



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

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]

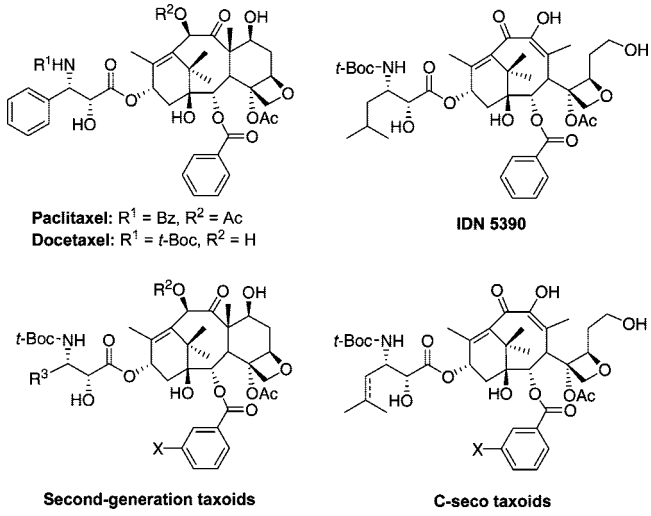


Figure 1

Design and Synthesis of Novel C-14-C-3′BzN-Linked Macrocyclic Taxoids

The first cryo-electron microscopy (cryo-EM) structure of paclitaxel-bound Zn^{2+}-stabilized $\alpha\beta$-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 ITUB 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 cytotoxicity[48−52] 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 2005[53] based on the two $^{13}C-^{19}F$ intramolecular distances of the microtubule-bound 2-(4-fluorobenzoyl)paclitaxel experimentally obtained by means of a REDOR NMR study,[54] MD

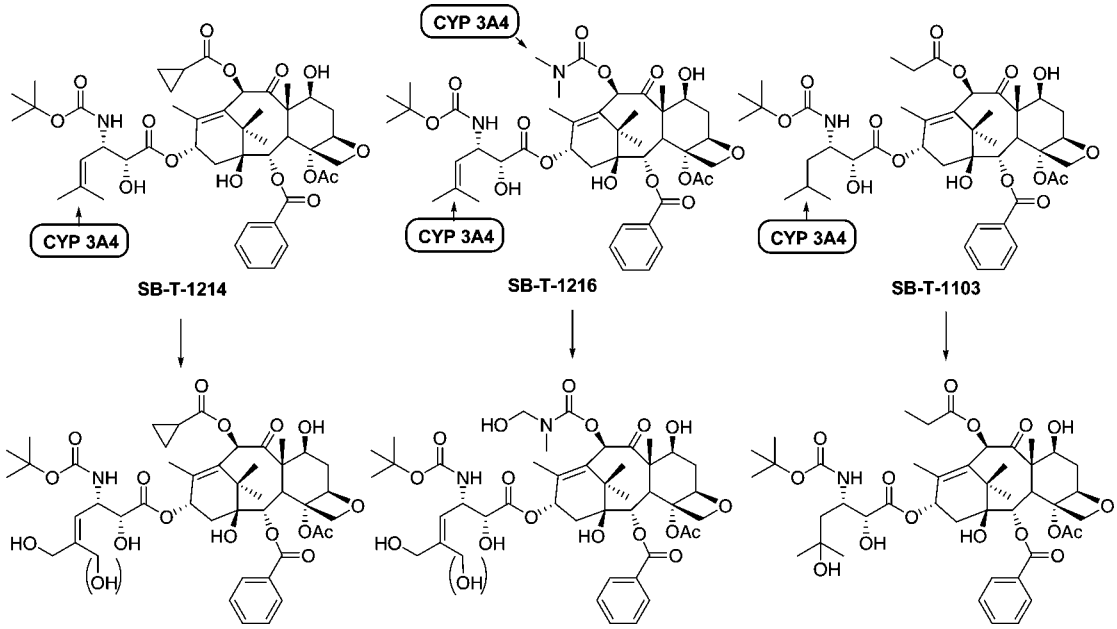
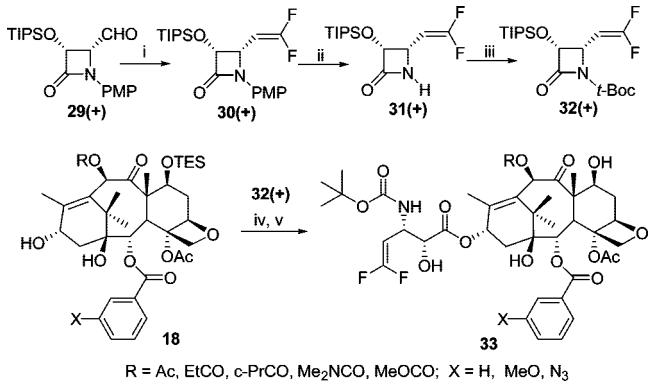


Figure 2. Primary sites of hydroxylation on the second-generation taxoids by the P450 family of enzymes.[41]

Scheme 8[a]



[a] Reagents and conditions: (i) CBr_2F_2, HMPT, Zn, THF, 84%; (ii) CAN, H_2O/CH_3CN, −15 °C, 92%; (iii) Boc_2O, Et_3N, DMAP, CH_2Cl_2, 96%; (iv) LiHMDS, THF, −40 °C, (v) HF/Py, Py/CH_3CN, overnight, 0 °C to RT, 57−91%.

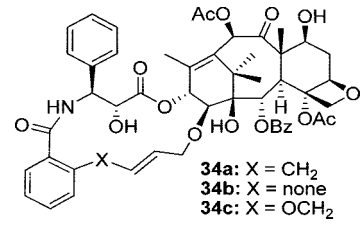


Figure 3. Designed novel C-14-C-3′BzN-linked macrocyclic taxoids.

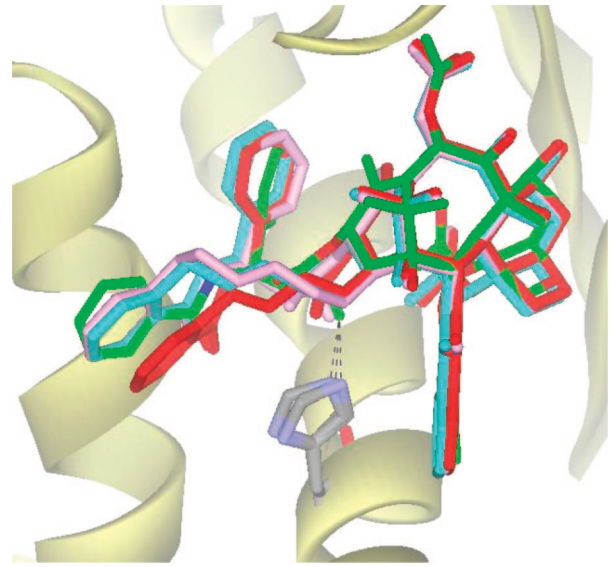


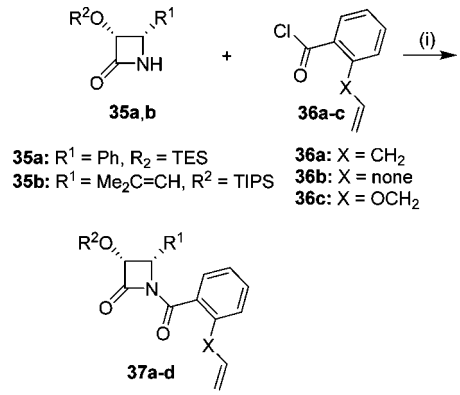
Figure 4. Overlays of REDOR-Taxol (green) with **34a** (cyan), **34b** (red), and **34c** (pink).

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 collaborators[56] and also accommodates highly active macrocyclic paclitaxel analogues designed based on the T-Taxol structure.[24] The C-2′-OH group interacts with His[227] 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 Arg[369] 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*-allyloxybaccatin **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]

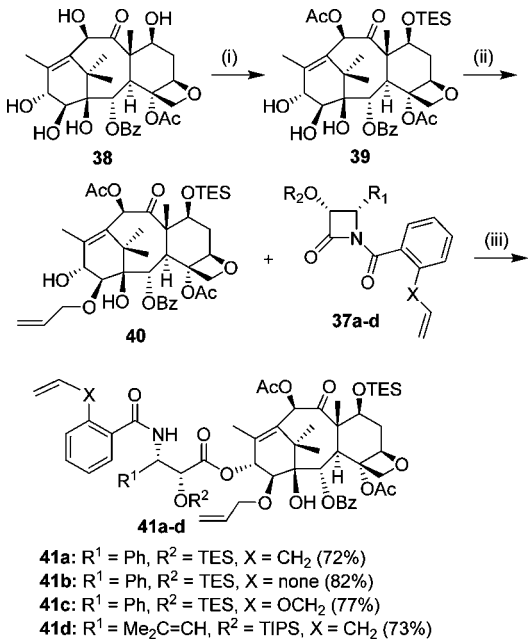
As Scheme 11 shows, the RCM reactions of paclitaxel-dienes **41a** and **41d** catalyzed by the "first-generation Grubbs catalyst" proceeded smoothly at room temperature to give the corresponding macrocyclic taxoids **42a** and **42d**, respectively. The subsequent deprotection of all silyl groups with HF-pyridine afforded the designed macrocyclic taxoids **34a** and **34d**, respectively, in high

Scheme 9[a]



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

Scheme 10[a]

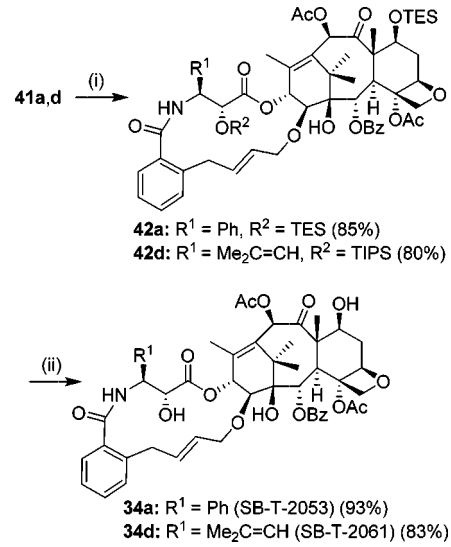


[a] Reagents and conditions: (i) (a) Ac_2O (10 equiv), $CeCl_3 \cdot 7H_2O$ (0.1 equiv), THF, RT, 2 h, (b) TESCl (3.0 equiv), imidazole (4.0 equiv), DMF, RT, 5 h (83% in 2 steps), (ii) allyl iodide (1.1 equiv), NaHMDS (1.1 equiv), DMF, −40 °C, 1 h (82%), (iii) **37a**−**d** (3.0 equiv), LiHMDS (1.5 equiv), THF, −30 to 0 °C, 2.5 h.

yields.[24] In both products, the *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).

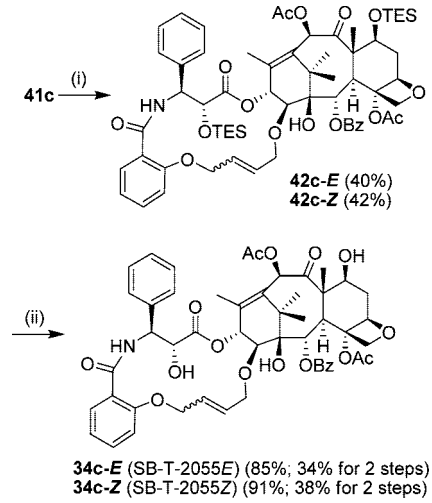
In contrast, the RCM reaction of paclitaxel-diene **41b** did not proceed as anticipated and gave **42b** unexpectedly, which was deprotected to afford macrocyclic taxoid **43b** in fairly good yield (Scheme 13).[24] The ^1H and ^{13}C NMR and 2D NMR analyses suggested that **43b** should possess a butenylene 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

Scheme 11[a]



[a] Reagents and conditions: (i) (a) $Cl_2Ru(=CHPh)(PCy_3)_2$ (0.2 equiv), CH_2Cl_2, overnight; (ii) HF/Py, Py, CH_3CN, RT, overnight.

Scheme 12[a]



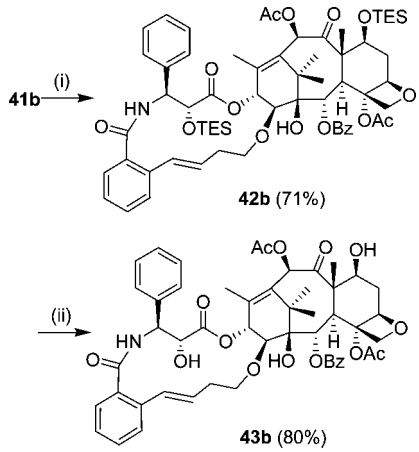
[a] Reagents and conditions: (i) (a) $Cl_2Ru(=CHPh)(PCy_3)_2$ (0.2 equiv), CH_2Cl_2, overnight, flash chromatography; (ii) HF/Py, Py, CH_3CN, RT, overnight.

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 and 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**−**o** 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 2 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

Scheme 13[a]



[a] Reagents and conditions: (i) (a) $Cl_2Ru(=CHPh)(PCy_3)_2$ (0.25 equiv × 3), CH_2Cl_2, reflux, 5 days; (ii) HF/Py, Py, CH_3CN, RT, overnight.

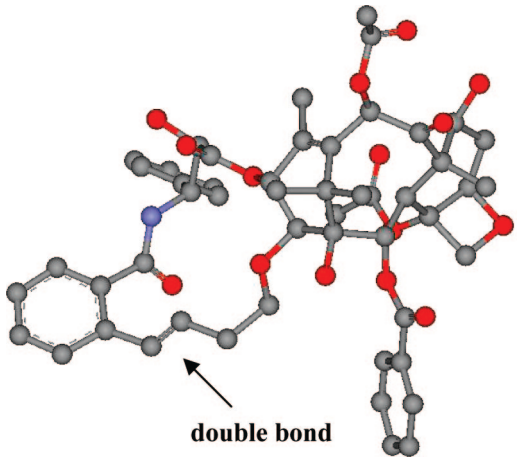


Figure 5. X-ray crystal structure of **43b** (SB-T-2054).

noteworthy feature. Taxoid **6d** shows almost no difference against drug-resistant and drug-sensitive cell lines with the R/S ratio of 1.2.

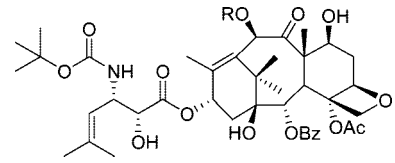
Taxoids **6a** and **6d**, with a benzoyl group and a 4-methoxybenzoyl group at C-10, respectively, possess highest potencies against LCC6-MDR, while taxoid **6k**, with a 4-methoxyphenylacetyl, is the least potent but possesses the highest potency against LCC6-WT. Taxoids with a substituted or unsubstituted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

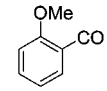
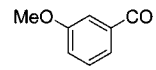
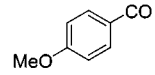
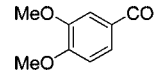
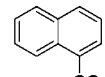
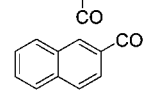
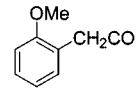
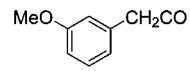
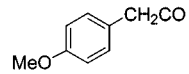
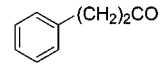
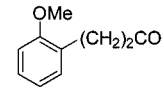
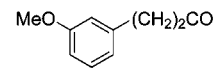
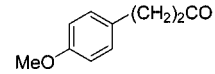
The activities of C-3′N-modified 10-propanoyl-taxoids are summarized in Table 6.[29] Most of these C-3′N-modified taxoids possess better potency against LCC6-WT and MCF7 cell lines and 1−2 orders of magnitude higher potency against drug-resistant LCC6-MDR and NCI/ADR cell lines, as compared with paclitaxel. Taxoids **15d** and **15e** exhibit high potency against these cell lines and possess cytotoxicity comparable to their parent taxoids SB-T-1213[7] 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 show 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 > N_3 > MeO ≫ CH_2=CH− against LCC6-WT (Pgp−) for the *meta*-substituted C2-

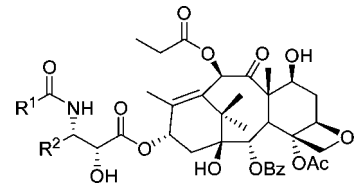
Table 5. Cytotoxicity of Second-Generation Taxoids with Modifications at C-10 (IC_{50} nM)[a]



taxoid	R	LCC6-WT[b]	LCC6-MDR[c]	R/S[d]
paclitaxel	--	3.1	346	112
6a	Bz	1.8	4.8	2.7
6b		2.1	5.8	2.7
6c		2.8	5.1	1.8
6d		3.8	4.7	1.2
6e		2.1	5.7	2.7
6f		5.3	15.2	2.9
6g		6.3	15.2	2.4
6h	Cbz	1.8	16.7	9.3
6i		2.3	13.8	6.0
6j		2.1	20.3	9.7
6k		1.4	38.4	27.4
6l		5.1	31.2	6.1
6m		1.5	5.0	3.3
6n		3.7	6.7	1.8
6o		1.7	4.9	2.9

[a] Concentration of compound that inhibits 50% (IC_{50}, nM) of the growth of human tumor cell line after a 72 h drug exposure. [b] LCC6-WT: human breast carcinoma cell line (Pgp−). [c] LCC6-MDR: *mdr1* transduced cell line (Pgp+). [d] Resistance factor = (IC_{50} for drug resistant cell line, R)/(IC_{50} for drug-sensitive cell line, S).

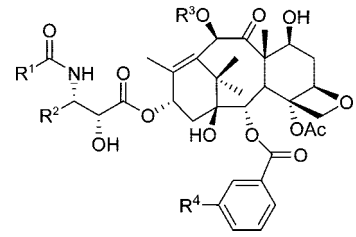
Table 6. Cytotoxicity of Second-Generation Taxoids with C-3′N Modifications (IC_{50} nM)[a]



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	*t*-Boc	2-Me-prop-1-enyl				0.18	4.0	22
SB-T-1103	*t*-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 footnote of Table 5. [b] See the footnote of Table 5. [c] See the footnote of Table 5. [d] See the footnote of Table 5. [e] MCF7: human breast carcinoma cell line. [f] NCI/ADR: multidrug-resistant human ovarian carcinoma cell line.

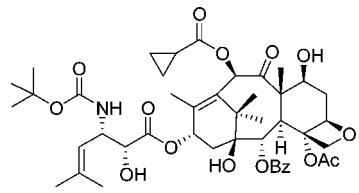
Table 7. Cytotoxicity of Second-Generation Taxoids with C-2-*meta* Modifications (IC_{50} nM)[a]



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	*t*-BuO	Ph	H	H	1.0	120	120	1.0	235	235
SB-T-1213	*t*-BuO	$Me_2C{=}CH-$	EtCO	H				0.18	4.0	22
SB-T-1103	*t*-BuO	Me_2CHCH_2-	EtCO	H				0.35	5.1	21
SB-T-1214	*t*-BuO	$Me_2C{=}CH-$	*c*-PrCO	H				0.20	3.9	20
12a	*t*-BuO	$Me_2C{=}CH-$	MeCO	Me	1.5	5.8	3.9	0.8	5.0	6.3
12b	*t*-BuO	$Me_2C{=}CH-$	MeCO	MeO	0.6	2.7	4.5	0.8	2.3	2.9
12c	*t*-BuO	$Me_2C{=}CH-$	EtCO	F	0.5	2.1	4.2			
12d	*t*-BuO	$Me_2C{=}CH-$	EtCO	Cl	0.8	1.3	1.6	–	–	–
12e	*t*-BuO	$Me_2C{=}CH-$	EtCO	N_3	0.9	1.2	1.3	0.9	1.1	1.2
12f	*t*-BuO	$Me_2C{=}CH-$	EtCO	$CH_2{=}CH-$	2.9	7.1	2.4			
12g	*t*-BuO	$Me_2C{=}CH-$	EtCO	MeO	1.0	0.9	0.90	0.36	0.33	0.92
12h	*t*-BuO	$Me_2C{=}CH-$	*c*-PrCO	MeO	1.0	2.9	2.9			
12i	*t*-BuO	$Me_2C{=}CH-$	MeOCO	MeO	0.6	1.6	2.7	0.4	1.4	3.5
12j	*t*-BuO	$Me_2C{=}CH-$	$PhCH_2OCO$	MeO	1.2	1.8	1.5	0.2	1.5	7.5
12k	*t*-BuO	$Me_2C{=}CH-$	2-MeO(C_6H_4)CO	MeO	0.4	0.9	2.3	1.1	3.3	3.0
12l	*t*-BuO	$Me_2C{=}CH-$	4-MeO(C_6H_4)CH_2CO	MeO	0.4	0.4	1.0	0.6	1.8	3.0
12m	*t*-BuO	$CH_2{=}CHCH_2$-	EtCO	MeO	1.2	8.4	7.0	0.8	8.7	10.9
12n	*t*-BuO	(*E*)-$CH_3CH{=}CH-$	EtCO	MeO	1.2	4.1	3.4			
12o	*t*-BuO	$CH_2{=}CH(CH_2)_2$-	EtCO	MeO	0.9	5.4	6.0	2.0	7.7	3.9
12p	*t*-BuO	(*S*)-2,2-Me_2-*c*-Pr-	EtCO	MeO	0.48	1.1	2.3	0.6	1.5	2.5
13c	*t*-BuO	Me_2CHCH_2-	EtCO	F	0.4	2.4	6.0			
13d	*t*-BuO	Me_2CHCH_2-	EtCO	Cl	0.8	2.9	3.6			
13e	*t*-BuO	Me_2CHCH_2-	EtCO	N_3	1.1	2.4	2.2	1.0	2.1	2.1
13g	*t*-BuO	Me_2CHCH_2-	EtCO	MeO	0.9	0.8	0.89	0.36	0.43	1.19
15h	*c*-Pr	$Me_2C{=}CH-$	EtCO	MeO	1.1	17.2	16	0.5	7.8	15.6
15i	*c*-Bu	$Me_2C{=}CH-$	EtCO	MeO	1.6	15	9.4	0.8	8.8	11
15j	*c*-Pentyl	$Me_2C{=}CH-$	EtCO	MeO	1.1	11	10	0.3	9.5	32
15k	*c*-Hexyl	$Me_2C{=}CH-$	EtCO	MeO	6.9	42	6.1	1.8	17.5	9.7
15l	*c*-Hex-O	$Me_2C{=}CH-$	EtCO	MeO	1.23	14.8	12.0	1.44	14	9.7
16h	*c*-Pr	Me_2CHCH_2-	EtCO	MeO	0.6	13	22	0.4	11.8	30
16k	*c*-Hexyl	Me_2CHCH_2-	EtCO	MeO	1.0	12	12	0.7	6.5	9.3
16m	*c*-Pent-O	Me_2CHCH_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. [c] 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 (IC_{50}, nM)[a]



SB-T-1214

taxoid	A-2780	1A9PTX10	R/S[b]	1A9PTX22	R/S[b]
paclitaxel	1.38 ± 0.05	532.95 ± 3.18	386	160.70 ± 14.70	116
SB-T-1214	0.44 ± 0.04	9.00 ± 0.77	20.4	3.94 ± 0.03	9.0
12g	0.76 ± 0.01	3.65 ± 0.21	4.8	3.88 ± 0.54	5.1
13g	0.25 ± 0.01	4.91 ± 0.53	19.6	2.10 ± 0.13	8.4

[a] See the footnote of Table 5. [b] See the footnote of Table 5.

Table 9. Cytotoxicity of **SB-T-1214** and **12g** against Pancreatic Cancer Cell Lines (IC_{50} 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

[a] See the footnote of Table 5.

benzoyl moiety, reflecting the taxoids's ability to bind microtubules. The potency order changes to MeO > N_3 > Cl > F ≫ CH_2═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**−**p** 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 2 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 μM concentration. A preliminary in vivo efficacy assay (20 mg/kg × 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]

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

treatment[a] (iv)	total dose (mg/kg)	growth delay[b] (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

[a] Treatment given iv to SCID mice on day 5 after DLD-1 human colon tumor implant and continued on days 8 and 11, with all drugs formulated in Tween/EtOH. [b] Based on comparison of each group vs control using the Cox-Mantel test. [c] Number of animals who either died or lost greater than 20% body weight. [d] SCID mice with no palpable tumors on day 167, the end of experiment.

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 × 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 (20 mg/kg × 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 Tables 11 and 12, respectively.[36] The IC_{50} 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 (2 orders of magnitude more potent than paclitaxel on average). The potency of 3′-CF_2H-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′-CF_3-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; that is, potency tends to increase in the order F < MeO < Cl < N_3. 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., IC_{50} (A2780TC3)/IC_{50} (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.

Table 11. In Vitro Cytotoxicity (IC_{50} nM)[a] of C-3′-CF_2H-taxoid (**21**)

taxoid	R	X	MCF7-S[b] (breast)	MCF7-R[c] (breast)	R/S[c]	LCC6-WT[b] (breast)	LCC6-MDR[c] (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_2N-CO	MeO	0.45	4.51	10	0.69	7.06	10	0.40	0.43
SB-T-12843-2	Me_2N-CO	F	0.52	8.13	16	0.69	10.6	15	0.20	0.35
SB-T-12843-3	Me_2N-CO	Cl	0.31	2.96	9.5	0.21	3.87	18	0.36	0.58
SB-T-12843-4	Me_2N-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 (IC_{50} nM)[a] of C-3′-CF_3-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_2NCO	MeO	0.57	1.84	3.2	0.28	4.48	16	0.35	0.68
SB-T-12823-2	Me_2NCO	F	0.32	2.64	8.3	0.32	5.57	17	0.5	0.76
SB-T-12823-3	Me_2NCO	Cl	0.12	1.02	8.5	0.27	2.55	9.4	0.42	0.45
SB-T-12823-4	Me_2NCO	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 (IC_{50} nM)[a] of C-seco-Fluorotaxoids

C-seco-taxoid	A2780wt[b]	A2780CIS[c]	A2780TOP[d]	A2780ADR[e]	A2780TC1[f]	A2780TC3[f]
paclitaxel	1.7	2.2	7.2	1239	10 027	17 800
IDN 5390	17.4	16.8	27.5	2617	2060	2237
28a (SB-CST-10104)	11.1	11.8	12.8	3726	1497	460
28b (SB-CST-10204)	6.1	4.9	6.9	2218	4454	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] Clone derived from chronic exposure of A2780 to paclitaxel and cyclosporine.

The C-3′-substitutents 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 the 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 Ca^{2+}-induced depolymerization.[24] This result also supports our observa-

Table 14. In Vitro Cytotoxicity (IC_{50} 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_2N-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 (IC_{50} 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*	1650	10 010	2067	2285	1475	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.

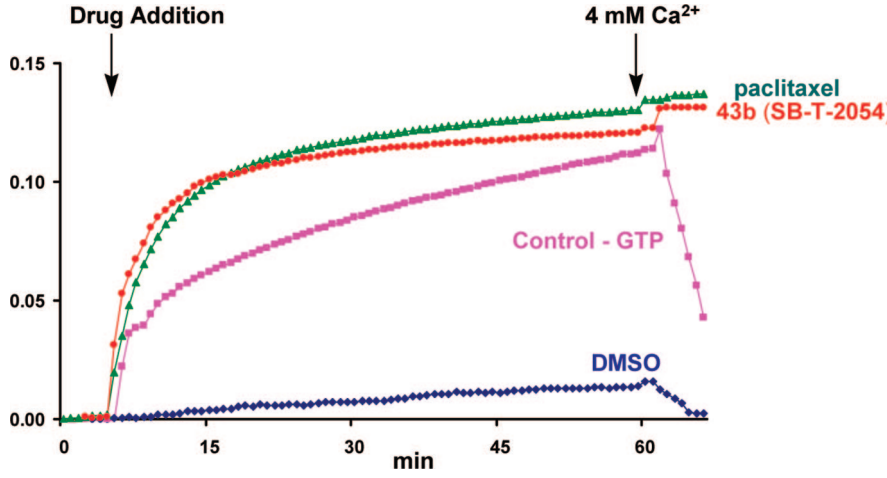


Figure 6. Tubulin polymerization with **43b** and paclitaxel: microtubule protein 1 mg/mL, 37 °C, GTP 1 mM, or drug 10 μM.

tion 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 multidrug 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.

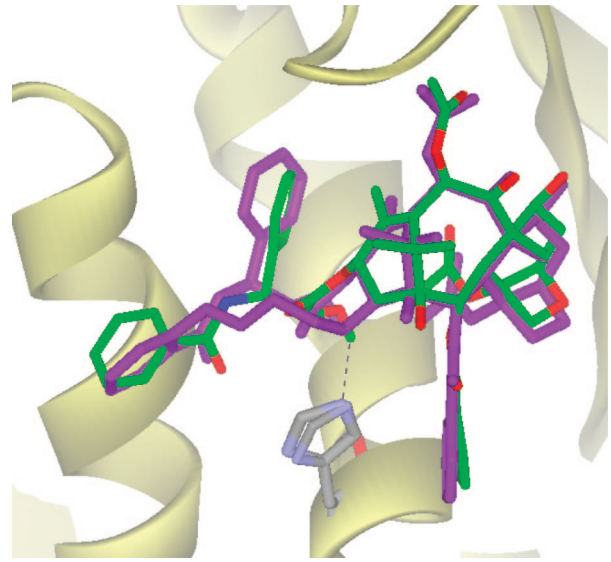


Figure 7. Overlays of REDOR-Taxol (green) with **43b** (SB-T-2054) (purple).

References and Notes

(1) Jemal, A.; Ward, E.; Hao, Y.; Thun, M. *J. Am. Med. Assoc.* **2005**, *294*, 1255–1259.
(2) Jemal, A.; Siegel, R.; Ward, E.; Murray, T.; Xu, J.; Smigal, C.; Thun, M. J. *CA: Cancer J. Clin.* **2006**, *56*, 106–130.
(3) Rowinsky, E. K. *Ann. Rev. Med.* **1997**, *48*, 353–374.
(4) Suffness, M. *Taxol: Science and Applications*; CRC Press: New York, 1995.
(5) Schiff, P. B.; Fant, J.; Horwitz, S. B. *Nature* **1979**, *277*, 665–667.
(6) Schiff, P. B.; Horwitz, S. B. *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 1561–1565.
(7) Ojima, I.; Slater, J. C.; Michaud, E.; Kuduk, S. D.; Bounaud, P.-Y.; Vrignaud, P.; Bissery, M.-C.; Veith, J.; Pera, P.; Bernacki, R. J. *J. Med. Chem.* **1996**, *39*, 3889–3896.
(8) Ojima, I.; Wang, T.; Miller, M. L.; Lin, S.; Borella, C.; Geng, X.; Pera, P.; Bernacki, R. J. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 3423–3428.
(9) Ojima, I.; Lin, S.; Wang, T. *Curr. Med. Chem.* **1999**, *6*, 927–954.
(10) Gottesman, M. M.; Fojo, T.; Bates, S. E. *Nat. Rev. Cancer* **2002**, *2*, 48–58.
(11) Sullivan, K. F. *Annu. Rev. Cell Biol.* **1988**, *4*, 687–716.
(12) Banerjee, A.; Roach, M. C.; Trcka, P.; Luduena, R. F. *J. Biol. Chem.* **1992**, *267*, 5625–5630.
(13) Panda, D.; Miller, H. P.; Banerjee, A.; Luduena, R. F.; Wilson, L. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 11358–11362.
(14) Kavallaris, M.; Kuo, D. Y. S.; Burkhart, C. A.; Regl, D. L.; Norris, M. D.; Haber, M.; Horwitz, S. B. *J. Clin. Invest.* **1997**, *100*, 1282–1293.
(15) Derry, W. B.; Wilson, L.; Khan, I. A.; Luduena, R. F.; Jordan, M. A. *Biochemistry* **1997**, *36*, 3554–3562.
(16) Ferlini, C.; Raspaglio, G.; Mozzetti, S.; Cicchillitti, L.; Filippetti, F.; Gallo, D.; Fattorusso, C.; Campiani, G.; Scambia, G. *Cancer Res.* **2005**, *65*, 2397–2405.
(17) Haber, M.; Burkhart, C. A.; Regl, D. L.; Madafiglio, J.; Norris, M. D.; Horwitz, S. B. *J. Biol. Chem.* **1995**, *270*, 31269–31275.
(18) Kavallaris, M. *Br. J. Cancer* **1999**, *80*, 1020–1025.
(19) Cottet, F.; Marull, M.; Lefebvre, O.; Schlosser, M. *Eur. J. Org. Chem.* **2003**, 1559–1568.
(20) Ojima, I.; Kuduk, S. D.; Slater, J. C.; Gimi, R. H.; Sun, C. M. *Tetrahedron* **1996**, *52*, 209–224.
(21) Ojima, I.; Kuduk, S. D.; Slater, J. C.; Gimi, R. H.; Sun, C. M.; Chakravarty, S.; Ourevitch, M.; Abouabdellah, A.; Bonnet-Delpon, D.; Begue, J.-P.; Veith, J. M.; Pera, P.; Bernacki, R. J. In *Biomedical Frontiers of Fluorine Chemistry*; Ojima, I., McCarthy, J. R., Welch, J. T., Eds.; ACS Symp. Ser. 639; American Chemical Society: Washington, D.C., 1996; p 228−243.
(22) Ojima, I.; Slater, J. C.; Pera, P.; Veith, J. M.; Abouabdellah, A.; Begue, J.-P.; Bernacki, R. J. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 133–138.
(23) Ojima, I.; Inoue, T.; Slater, J. C.; Lin, S.; Kuduk, S. C.; Chakravarty, S.; Walsh, J. J.; Gilchrist, L.; McDermott, A. E.; Cresteil, T.; Monsarrat, B.; Pera, P.; Bernacki, R. J. In *Asymmetric Fluoroorganic Chemistry: Synthesis, Application, and Future Directions*; Ramachandran, P. V., Ed.; ACS Symp. Ser. 746;American Chemical Society: Washington, D.C., 1999; pp 158−181.
(24) Sun, L.; Geng, X.; Geney, R.; Li, Y.; Simmerling, C.; Li, Z.; Lauher, J. W.; Xia, S.; Horwitz, S. B.; Veith, J. M.; Pera, P.; Bernacki, R. J.; Ojima, I. *J. Org. Chem.* **2008**, *73*, 9584–9593.
(25) Georg, G. I.; Harriman, G. C. B.; Vander Velde, D. G.; Boge, T. C.; Cheruvallath, Z. S.; Datta, A.; Hepperle, M.; Park, H.; Himes, R. H.; Jayasinghe, L. In *Taxane Anticancer Agents: Basic Science and Current Status*; Georg, G. I., Chen, T. T., Ojima, I., Vyas, D. M., Eds.; American Chemical Society: Washington, D.C., 1995; pp 217−232.
(26) Ojima, I.; Zucco, M.; Duclos, O.; Kuduk, S. D.; Sun, C.-M.; Park, Y. H. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 2479–2482.
(27) Ojima, I.; Duclos, O.; Kuduk, S. D.; Sun, C.-M.; Slater, J. C.; Lavelle, F.; Veith, J. M.; Bernacki, R. J. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 2631–2634.
(28) Ojima, I.; Slater, J. S.; Kuduk, S. D.; Takeuchi, C. S.; Gimi, R. H.; Sun, C.-M.; Park, Y. H.; Pera, P.; Veith, J. M.; Bernacki, R. J. *J. Med. Chem.* **1997**, *40*, 267–278.
(29) Ojima, I.; Chen, J.; Sun, L.; Borella, C. P.; Wang, W.; Miller, M. L.; Lin, S.; Geng, X.; Kuznetsova, L.; Qu, C.; Gallager, D.; Zhao, X.; Zanardi, I.; Xia, S.; Horwitz, S. B.; Mallen-St, J.; Guerriero, J. L.; Bar-Sagi, D.; Veith, J. M.; Pera, P.; Bernacki, R. J. *J. Med. Chem.* **2008**, *51*, 3203–3221.
(30) Ojima, I.; Sun, C. M.; Zucco, M.; Park, Y. H.; Duclos, O.; Kuduk, S. D. *Tetrahedron Lett.* **1993**, *34*, 4149–4152.
(31) Marder-Karsenti, R.; Dubois, J.; Bricard, L.; Guénard, D.; Guéritte-Voegelein, F. *J. Org. Chem.* **1997**, *62*, 6631–6637.
(32) Chen, S. H.; Kant, J.; Mamber, S. W.; Roth, G. P.; Wei, J.; Marshall, D.; Vyas, D.; Farina, V. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 2223–2228.
(33) Ojima, I.; Lin, S. *J. Org. Chem.* **1998**, *63*, 224–225.
(34) Chen, J.; Kuznetsova, L. V.; Ungreanu, I. M.; Ojima, I. In *Enantioselective Synthesis of β-Amino Acids*, 2nd ed.; Juaristi, E., Soloshonok, V., Eds.; John Wiley: New York, 2005; pp 447−476.
(35) Ojima, I. *Acc. Chem. Res.* **1995**, *28*, 383−389, andreferences therein.
(36) Kuznetsova, L. V.; Pepe, A.; Ungureanu, I. M.; Pera, P.; Bernacki, R. J.; Ojima, I. *J. Fluor. Chem.* **2008**, *129*, 817–828.
(37) Ojima, I.; Wang, T.; Miller, M. L.; Lin, S.; Borella, C. P.; Geng, X.; Pera, P.; Bernacki, R. J. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 3423–3428.
(38) Appendino, G.; Danieli, B.; Jakupovic, J.; Belloro, E.; Scambia, G.; Bombardelli, E. *Tetrahedron Lett.* **1997**, *38*, 4273–4276.
(39) Appendino, G.; Noncovich, A.; Bettoni, P.; Dambruoso, P.; Sterner, O.; Fontana, G.; Bombardelli, E. *Eur. J. Org. Chem.* **2003**, 4422–4431.
(40) Gut, I.; Ojima, I.; Vaclavikova, R.; Simek, P.; Horsky, S.; Soucek, P.; Kondrova, E.; Kuznetsova, L. V.; Chen, J. *Xenobiotica* **2006**, *36*, 772–792.
(41) Vuilhorgne, M.; Gaillard, C.; Sanderlink, G. J.; Royer, I.; Monsarrat, B.; Dubois, J.; Wright, M. In *Taxane Anticancer Agents: Basic Science and Current Status*; Georg, G. I., Chen, T. T., Ojima, I., Vyas, D. M., Eds.; ACS Symp. Ser. 583; American Chemical Society: Washington, D.C., 1995; pp 98−110.
(42) Ojima, I.; Kuznetsova, L. V.; Sun, L. In *Current Fluoroorganic Chemistry. New Synthetic Directions, Technologies, Materials and Biological Applications*; Soloshonok, V., Mikami, K., Yamazaki, T., Welch, J. T., Honek, J., Eds.; ACS Symp. Ser. 949; American Chemical Society/Oxford University Press: Washington, D.C., 2007; pp 288−304.
(43) Ojima, I.; Habus, I.; Zhao, M.; Zucco, M.; Park, Y. H.; Sun, C. M.; Brigaud, T. *Tetrahedron* **1992**, *48*, 6985–7012.
(44) Ojima, I.; Kuduk, S. D.; Chakravarty, S. In *Advanced Medicinal Chemistry*; Maryanoff, B. E., Reitz, A. B., Eds.; JAI Press: Greenwich, CT, 1998; Vol. 4, pp 69−124.
(45) Nogales, E.; Wolf, S. G.; Downing, K. H. *Nature* **1998**, *391*, 199–203.
(46) Lowe, J.; Li, H.; Downing, K. H.; Nogales, E. *J. Mol. Bol.* **2001**, *313*, 1045–1057.
(47) Snyder, J. P.; Nettles, J. H.; Cornett, B.; Downing, K. H.; Nogales, E. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 5312–5316.
(48) Kingston, D. G. I.; Bane, S.; Snyder, J. P. *Cell Cycle* **2005**, *4*, 279–289.
(49) Ganesh, T.; Guza, R. C.; Bane, S.; Ravindra, R.; Shanker, N.; Lakdawala, A. S.; Snyder, J. P.; Kingston, D. G. I. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 10006–10011.
(50) Querolle, O.; Dubois, J.; Thoret, S.; Roussi, F.; Gueritte, F.; Guenard, D. *J. Med. Chem.* **2004**, *47*, 5937–5944.
(51) Ganesh, T.; Yang, C.; Norris, A.; Glass, T.; Bane, S.; Ravindra, R.; Banerjee, A.; Metaferia, B.; Thomas, S. L.; Giannakakou, P.; Alcaraz, A. A.; Lakdawala, A. S.; Snyder, J. P.; Kingston, D. G. I. *J. Med. Chem.* **2007**, *50*, 713–725.
(52) Larroque, A.-L.; Dubois, J.; Thoret, S.; Aubert, G.; Chiaroni, A.; Gueritte, F.; Guenard, D. *Bioorg. Med. Chem.* **2007**, *15*, 563–574.
(53) Geney, R.; Sun, L.; Pera, P.; Bernacki, R. J.; Xia, S.; Horwitz, S. B.; Simmerling, C. L.; Ojima, I. *Chem. Biol.* **2005**, *12*, 339–348.
(54) Li, Y.; Poliks, B.; Cegelski, L.; Poliks, M.; Cryczynski, A.; Piszcek, G.; Jagtap, P. G.; Studelska, D. R.; Kingston, D. G. I.; Schaefer, J.; Bane, S. *Biochemistry* **2000**, *39*, 281–291.
(55) Rao, S.; He, L.; Chakravarty, S.; Ojima, I.; Orr, G. A.; Horwitz, S. B. *J. Biol. Chem.* **1999**, *274*, 37990–37994.
(56) Paik, Y.; Yang, C.; Metaferia, B.; Tang, S.; Bane, S.; Ravindra, R.; Shanker, N.; Alcaraz, A. A.; Johnson, S. A.; Schaefer, J.; O'Connor, R. D.; Cegelski, L.; Snyder, J. P.; Kingston, D. G. I. *J. Am. Chem. Soc.* **2007**, *129*, 361–370.
(57) Giannakakou, P.; Sackett, D. L.; Kang, Y.-K.; Zhan, Z.; Buters, J. T. M.; Fojo, T.; Poruchynsky, M. S. *J. Biol. Chem.* **1997**, *272*, 17118–17125.
(58) Lage, H.; Dietel, M. *J. Cancer Res. Clin. Oncol.* **2002**, *128*, 349–357.
(59) O'Driscoll, L.; Walsh, N.; Larkin, A.; Ballot, J.; Ooi, W. S.; Gullo, G.; O'Connor, R.; Clynes, M.; Crown, J.; Kennedy, S. *Anticancer Res.* **2007**, *27*, 2115–2120.
(60) Zhou, J.; Liu, M.; Aneja, R.; Chandra, R.; Lage, H.; Joshi, H. C. *Cancer Res.* **2006**, *66*, 445–452.
(61) Mallen-St. Clair, J.; Curato, J.; Chen, J.; Ojima, I.; Karpeh, M.; Bar-Sagi, D. *Abstracts of the American Association for Cancer Research 97th Annual Meeting*, Washington, DC, April 1−5 2006, p 1963.
(62) Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S.; Boyd, M. R. *J. Natl. Cancer Inst.* **1990**, *82*, 1107–1112.

NP8006556