Development of Next Generation Taxoids by Iwao Ojima

2022.5.28. Literature Session

M2 Yosuke Nakata

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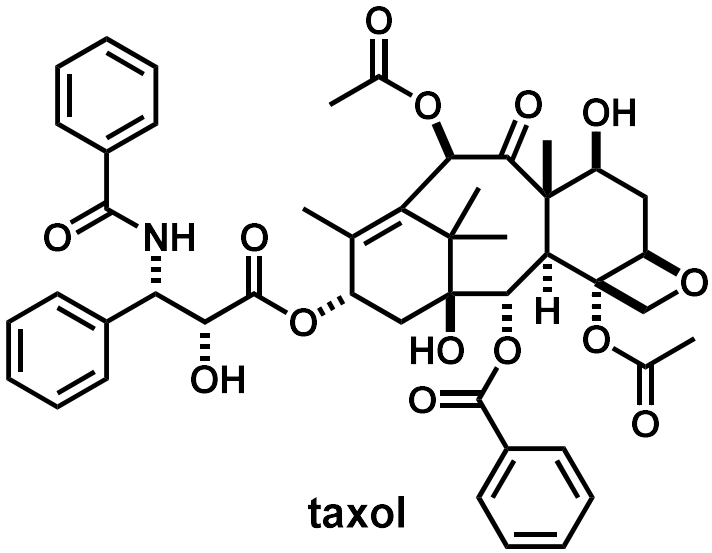
Taxol



taxol

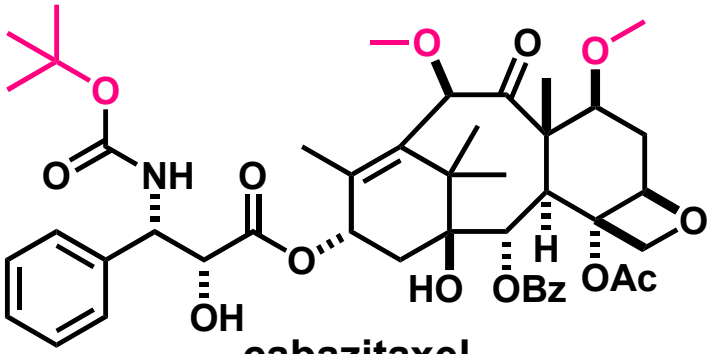
anticancer drug for many types of cancer
(ovarian, breast, lung…etc)

1960s isolated from the bark of *Taxus brevifolia*

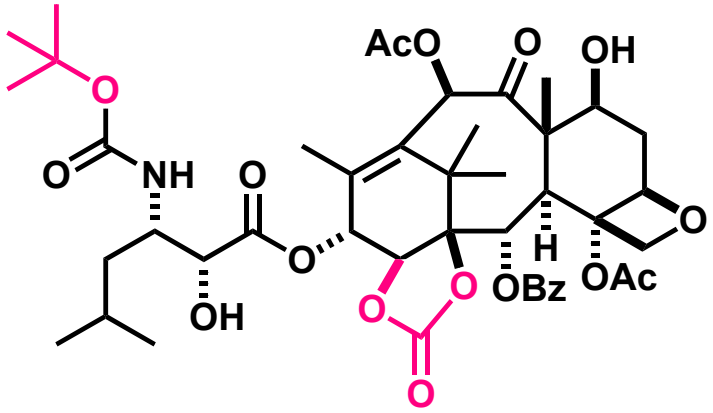
1971 structure determined[1)]
- **baccatin core**
  tricyclo[9.3.1.$0^{3,8}$]pentadecane
  9 chiral centers, oxetane, bridgehead olefin
- **β-amino acid side chain**

1992 approved as ovarian cancer reagent (FDA)[2)]

Several taxol derivatives (taxoids) have been approved or tested on clinical trials.



cabazitaxel
(anti prostate cancer)



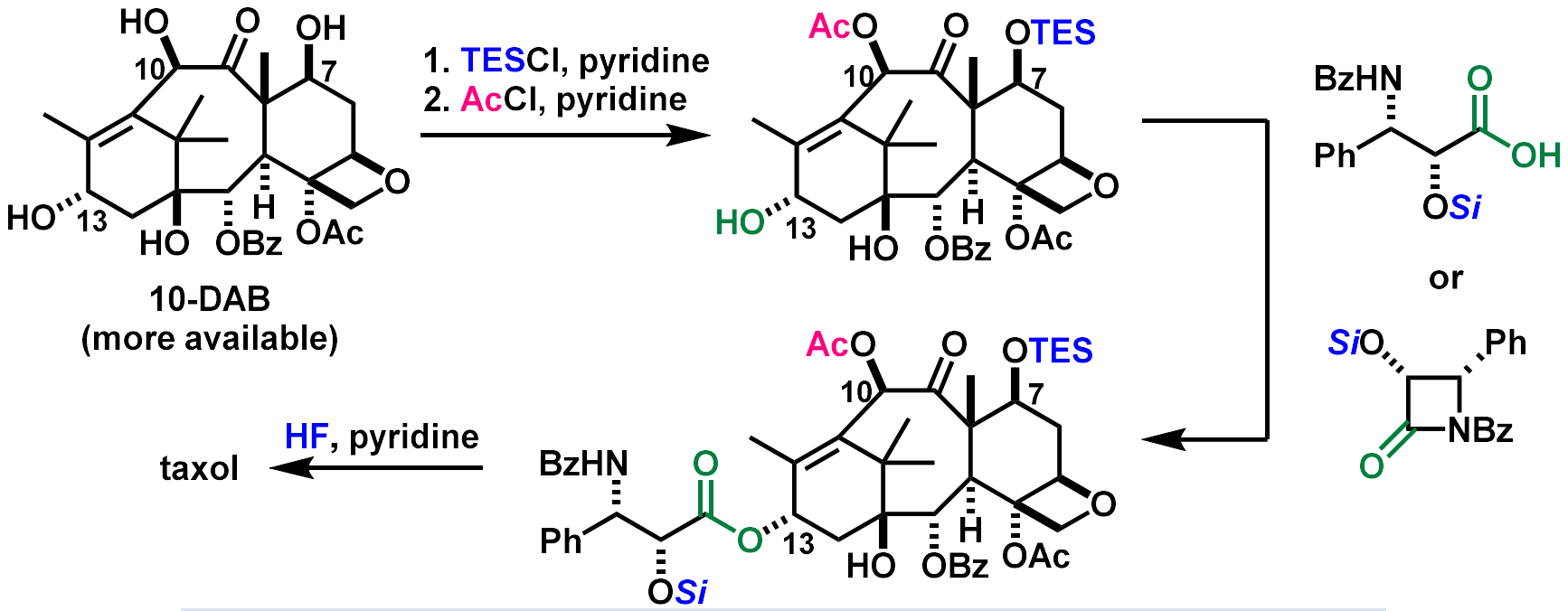
ortataxel (phase II)

1) Wani, C. M.; Taylor, L. H.; Wall, E. M.; Coggon, P.; McPhail, T. A. *J. Am. Chem. Soc.* **1971**, *93*, 2325.
2) Menzin, A. W.; King, S. A.; Aikins, J. K.; Mikuta, J. J.; Rubin, S. C. *Gynecol. Oncol.* **1994**, *54*, 103.

Synthesis

The amount of taxol isolated from *Taxus brevifolia* is scarce (10g taxol / 1200 kg the bark[1])
→ Synthetic strategy from available compounds should be constructed.

Semi-synthesis from 10-deacetylbaccatin (10-DAB)

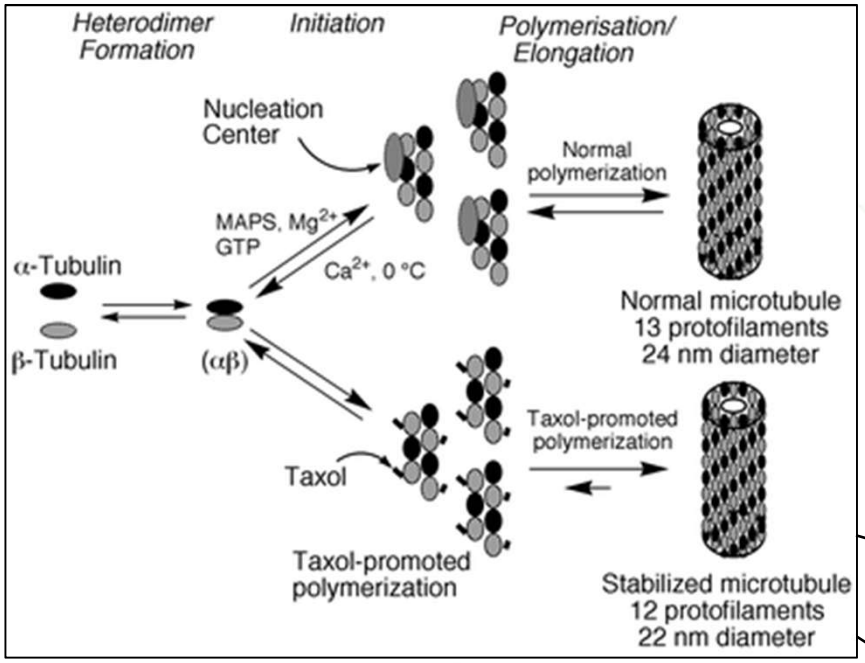


Asymmetric synthesis of β-lactam (or β-amino acid) has been developed.

Total synthesis : focused on the construction of complicated baccatin core
see also 201031_LS_Yusuke_Imamura

1) Jordan; G.; Vivien, W. (2001) *The Story of Taxol: Nature and Politics in the Pursuit of an Anti-Cancer Drug.* Cambridge University Press. p. 81.

Biological Activity



biological target : β-tubulin (βI)[1)]
Mechanisms of action:

- **promote assembly of microtubes**
- **stabilize microtube fibers**

Mode of action[2)]: G2/M phase arrest → apoptosis

1) Schiff, P. B.; Fant, J.; Horwitz, S. B. *Nature* **1979**, *277*, 665. 2) Ganguly, A.; Yang, H.; Cabral, F. *Mol. Cancer* **2010**, *9*, 1. (pictures) (a) Kingston, D. G. I. *Chem. Commun.* **2001**, 867. (b) Skubnik, J.; Pavlickova. V.; Ruml, T.; Rimpelova, S. *Plants* **2021**, *10*, 569.

Problems of Taxol Treatment[1)]

1. side effect:

neutropenia, cardiac rhythm disturbances
nausea, vomiting, diarrhea, alopecia of the scalp, inflammation
peripheral neuropathy (see also 220113_LS_Hiroaki_Itoh)

These effects maybe due to the inhibition of cell-cycle progression in the G_2 and M phases, but detailed mechanisms were not resolved.

2. drug resistance:

multi-drug resistance
(overexpression of P-glycoprotein (Pgp): membrane efflux pump)

tubulin diversification (β-tubulin isotypes, point mutation)

cancer stem cells (CSCs)

To overcome these problems, new toxoids with
- **improved anticancer activity (suppressing side effect by reducing dose)**
- **enhanced activity against taxol resistant cells**

have been developed.

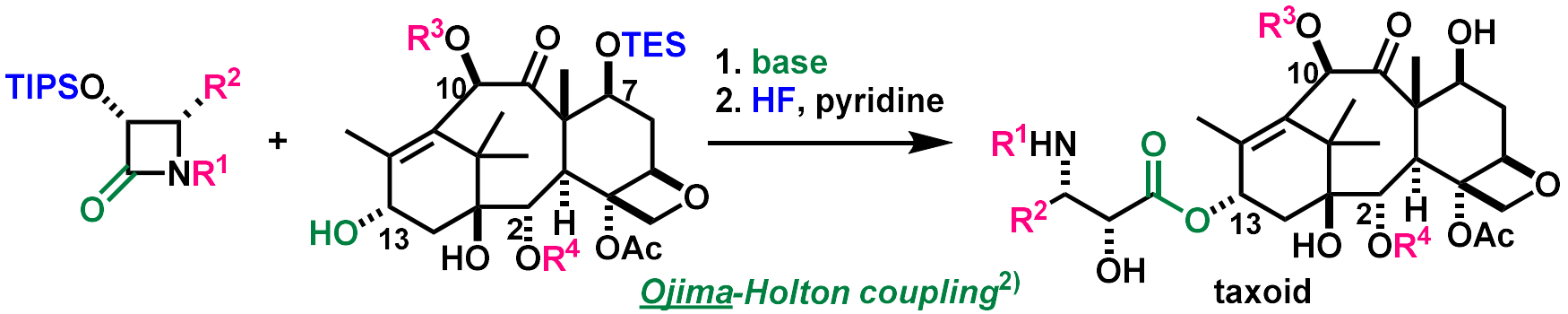
1) Rowinsky, K. E. *Annu. Rev. Med.* **1997**, *48*, 353.

Prof. Iwao Ojima

Prof. Iwao Ojima[1)]

B.S.: The University of Tokyo (1968)
Ph.D.: The University of Tokyo (1973) (Prof. Naoki Inamoto)
Senior Research Fellow and Group Leader: Sagami Institute of Chemical Research (1973-1983)
Associate Professor (1983), Professor (1984), Leading Professor (1991), Distinguished Professor (1995), Chairman (1997): State University of New York at Stony Brook
Director: Institute of Chemical Biology and Drug Discovery, Stony Brook. (2003-)
President: National Academy of Inventors Stony Brook University Chapter. (2016-)
Visiting Professor: Université Claude Bernard Lyon I (1989), The University of Tokyo (1996), The Scripps Research Institute (1997), Université de Paris XI (1997)

Greatly contributed to study of SAR, activity improvement, drug delivery…etc of taxol and its derivatives.



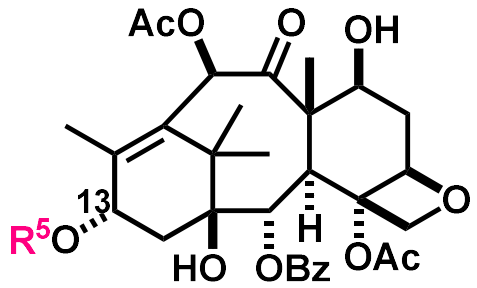
Ojima-Holton coupling[2)]

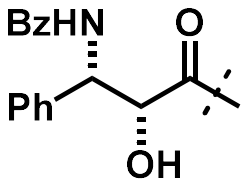
1) https://www.stonybrook.edu/commcms/ojima_group/profojima.html
2) Ojima, I. H. I.; Zhao, M.; Georg, G. I.; Jayasinghe, R. J. *J. Org. Chem.* **1991**, *56*, 1681.
3) Ojima, I.; Wang, X.; Jing, Y.; Wang, C. *J. Nat. Prod.* **2018**, *81*, 703.

Contents

Initial SAR Study

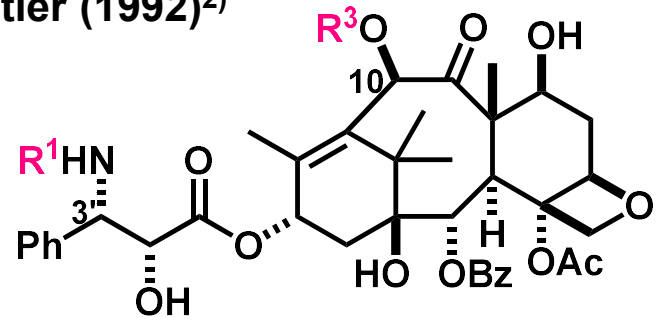
Kingston (1982)[1)]



	R^5	Growth inhibition (IC_{50}[μM], J774.2)
Taxol		0.08
baccatin III	H	> 20

C-13 side chain was necessary for the anticancer activity.

Potier (1992)[2)]



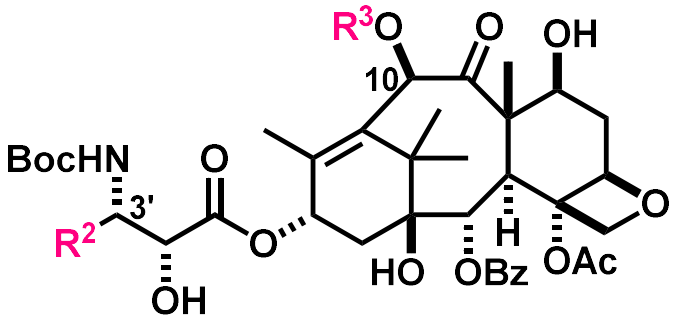
	R^1	R^3	Cytotoxicity (IC_{50}[μM], P388)
taxol	Bz	Ac	0.27
docetaxel	Boc	H	0.13

Docetaxel, more potent anticancer drug, was developed by modification of 3'N amide and C10-OH.

Activity improvement by modification of C13 side chain and C10-OH was suggested.

1) (a) Parness, J.; Kingston, D. G. I.; Powell, R. G.; Harracksingh, C.; Horwitz, S. B. *Biochem. Biophys. Res. Commun.* **1982**, *105*, 1082. 2) (a) Gueritte-Voegelein, F.; Guenard, D.; Lavelle, F.; Goff, L. T. M.; Mangatal, L.; Potier, P. *J. Med. Chem.* **1991**, *34*, 3, 99.

2nd Generation Taxoids



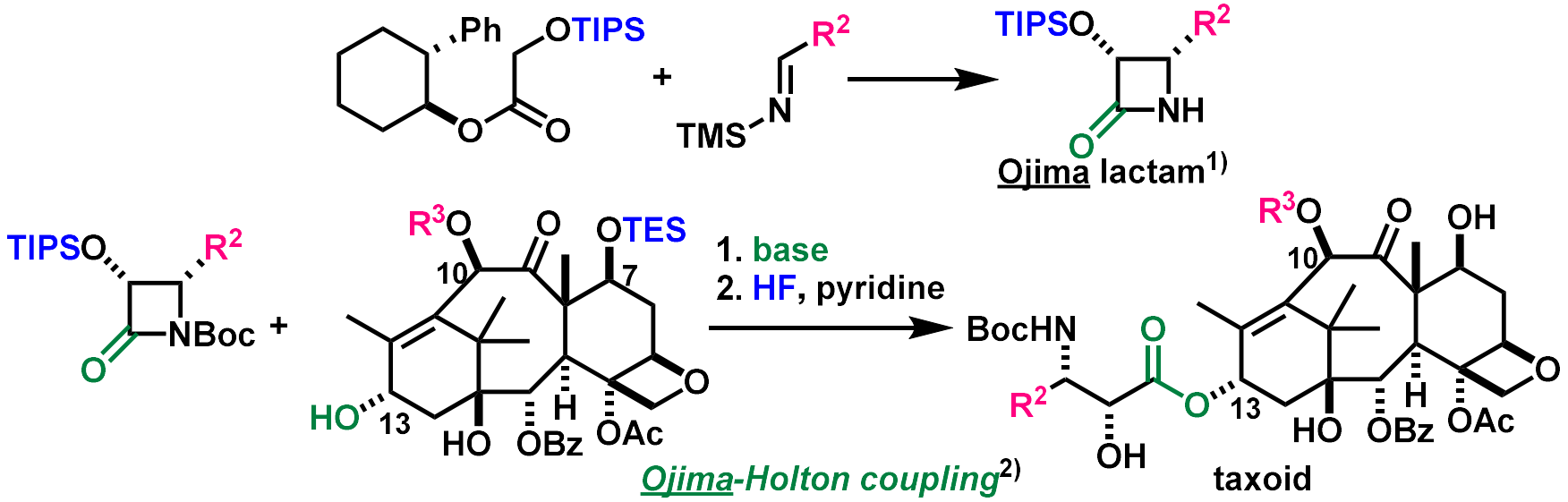
diversification of C3' and C10-OH

taxol: R^2 = Ph, R^3 = Ac

docetaxel: R^2 = Ph, R^3 = H

2nd generation[1]: R^2 = alkyl, alkenyl, R^3 = acyl

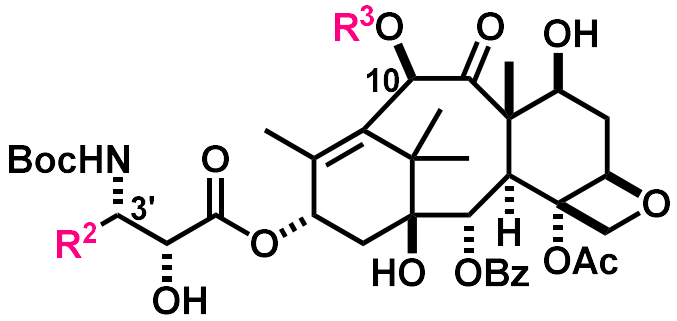
Synthesis of 2nd generation taxoids

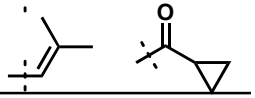


Asymmetric synthesis of β-lactam (Ojima lactam) and Ojima-Holton coupling were key reactions.

1) Ojima, I.; Slater, J. C.; Michaud, E.; Kuduk, S. D.; Bounaud, P.-Y.; Vrignaud, P.; Bissery, M. C.; Veith, J. M.; Pera, P.; Bernacki, R. J. *J. Med. Chem.* **1996**, *39*, 3889. 2) Ojima, I. H. I.; Zhao, M.; Georg, G. I.; Jayasinghe, R. J. *J. Org. Chem.* **1991**, *56*, 1681.

SAR of 2nd Generation Taxoids



	R²	R³
SB-T-1102		Ac
SB-T-1212		Ac
SB-T-1214		

cytotoxicity IC_{50}[nM][2)]	A121 (ovarian)	A549 (NSCL)	HT-29 (colon)	MCF-7 (breast)	MCF7-R (drug resistant)
taxol	6.3	3.6	3.6	1.7	299
docetaxel	1.2	1.0	1.2	1.0	235
SB-T-1102	3.8	0.98	3.2	4.0	36
SB-T-1212	0.46	0.27	0.63	0.55	12
SB-T-1214	0.26	0.57	0.36	0.20	2.1

C3' modification improved the anticancer activity.

C10-OH modification enhanced the cytotoxicity against drug resistant cells.

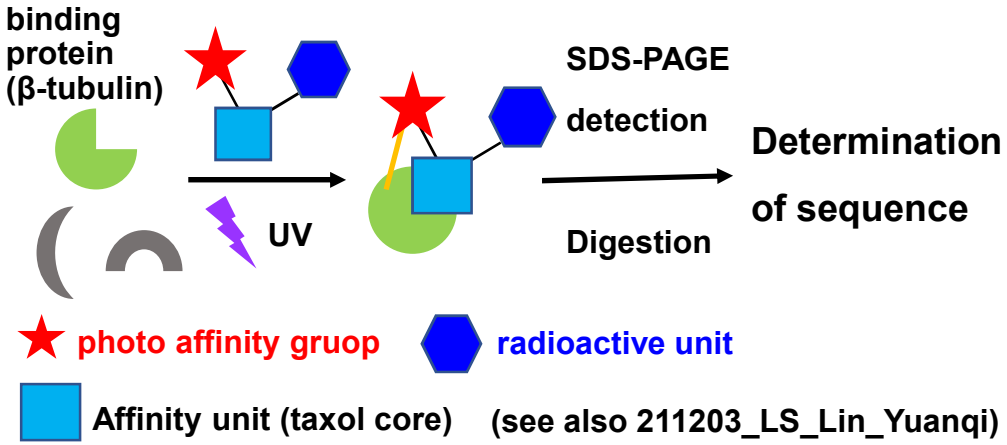
Binding mode analysis was needed for detailed explanation of these enhanced activities.

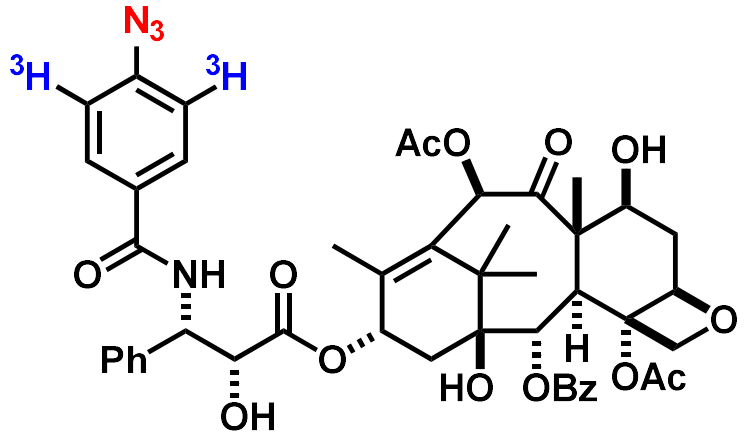
1) (a) Ojima, I.; Duclos, O.; Zucco, M.; Bissery, M.-C.; Combeau, C.; Vrignaud, P.; Riou, J. F.; Lavelle, F. *J. Med. Chem.* **1994**, *37*, 2602. (b) Ojima, I.; Duclos, O.; Kuduk, S. D.; Sun, C.-M.; Slater, J. C.; Lavelle, F.; Veith, J. M.; Bernacki, R. J. Bioorg. Med. Chem. Lett. 1994, 4, 2631. 2) Ojima, I.; Slater, J. C.; Michaud, E.; Kuduk, S. D.; Bounaud, P.-Y.; Vrignaud, P.; Bissery, M. C.; Veith, J. M.; Pera, P.; Bernacki, R. *J. J. Med. Chem.* **1996**, 39, 3889.

Binding Site Analysis by Photo Affinity Labeling (PAL)

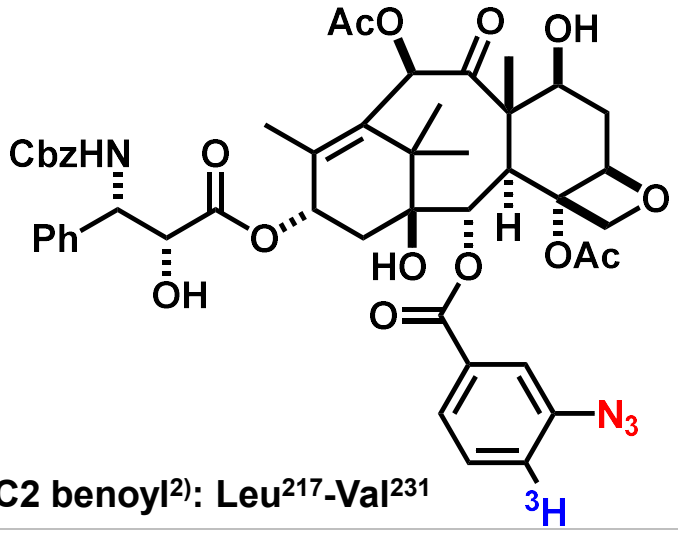
PAL and sequence analysis:

detect the residues of protein close to the affinity unit

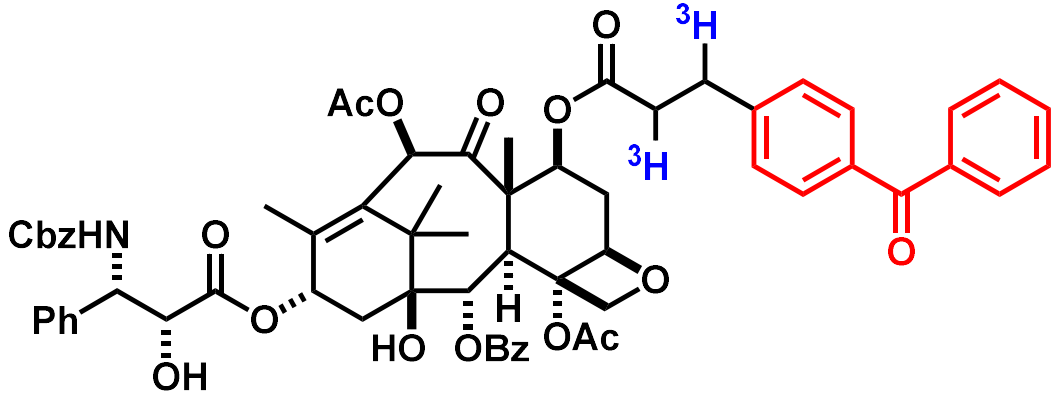




C3' benzamide[1]: Met[1]-Asp[31]



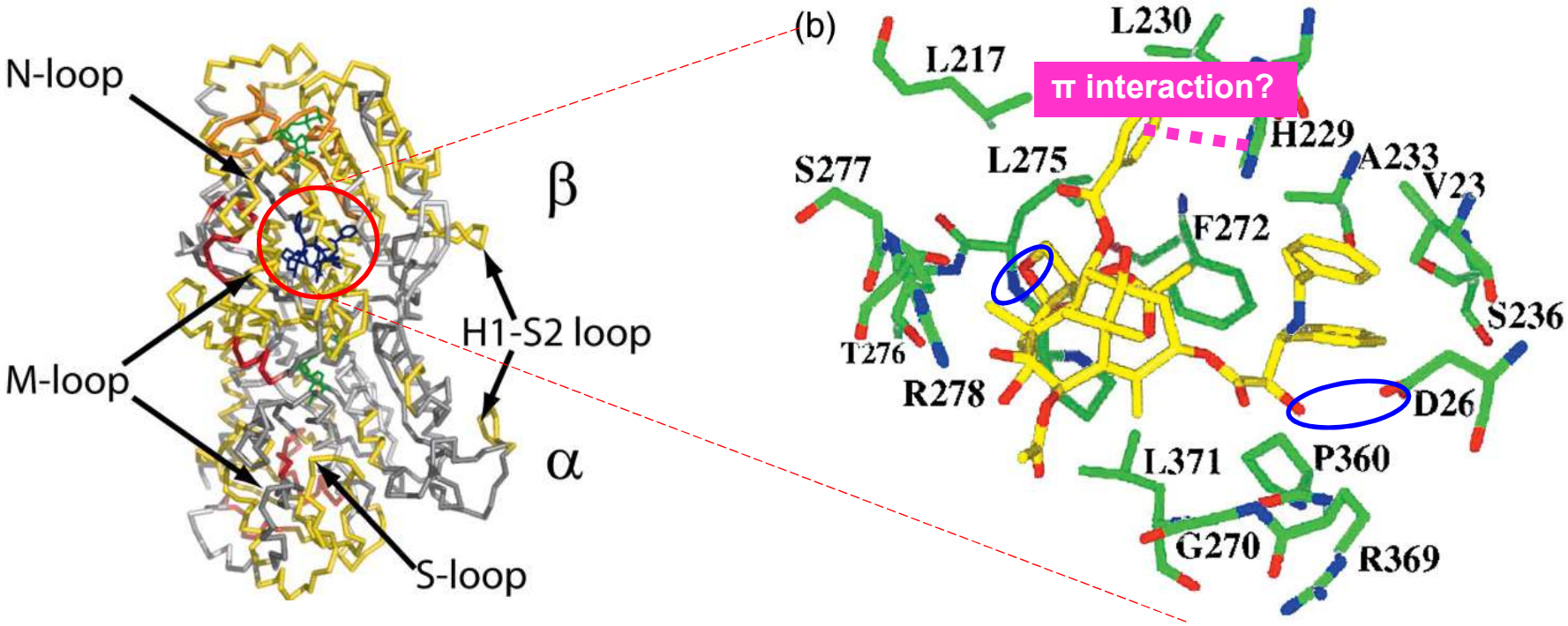
C2 benoyl[2]: Leu[217]-Val[231]



C7 hydroxy[3]: Arg[277]-Gln[293]

1) Rao, S.; Orr, G. A.; Chaudhary, A. G.; Kingston, D. G. I.; Horwitz, S. B. *J. Biol. Chem.* **1995**, *270*, 20235. 2) Rao, S.; Krauss, N. E.; Heerding, J. M.; Swindell, C. S.; Ringel, I.; Orr, G. A.; Horwitz, S. B. *J. Biol. Chem.* **1994**, 269, 3132. 3) Rao, S.; He, L.; Chakravarty, S.; Ojima, I.; Orr, G. A.; Horwitz, S. B. *J. Biol. Chem.* **1999**, *274*, 37990.

Binding Site Analysis by Docking Calculation



3.5 Å X-ray crystallography (stabilized by Zn^{2+})

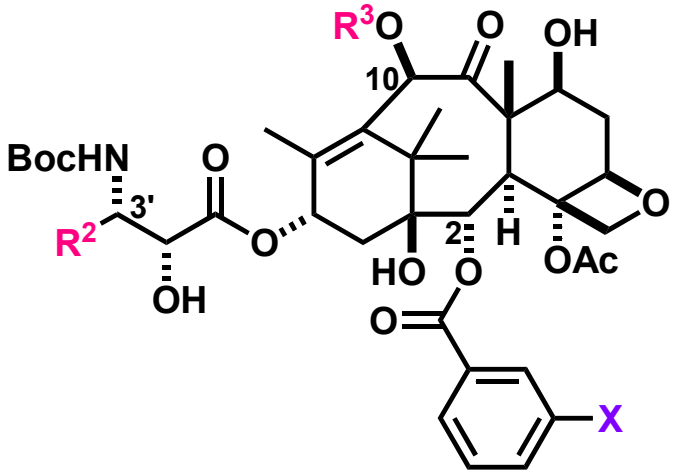
Possible hydrogen bonding was suggested.[1)]

(C2'-OH to Asp^{26}, C5-oxetane to Leu^{275}, C10 carbonyl to Arg^{284})

Involvement of C2 benzoyl moiety by the interaction with His^{229} was also suggested.

1) Löwe, J.; Li, H.; Downing, K.; Nogales, E. *J. Mol. Biol.* **2001**, *313*, 1045. 2) Xiao, H.; Verier-Pinard, P.; Fernandez-Fuentes, N.; Burd, B.; Angeletti, R.; Andras, F.; Horwitz, B. S.; Orr, A. G. *Proc. Natl. Acad. Sci. USA* **2005**, *103*, 10166.

3rd Generation Taxoids



diversification of C-3', C10, and C2

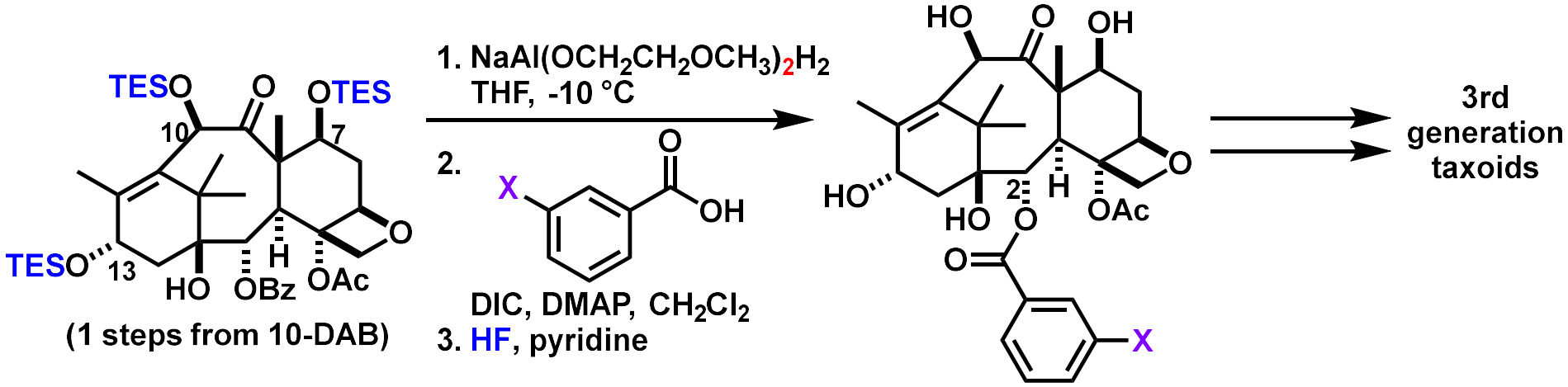
taxol: R^2 = Ph, R^3 = Ac, X = H

docetaxel: R^2 = Ph, R^3 = H, X = H

3rd generation[1]: R^2 = alkyl, alkenyl, R^3 = acyl,

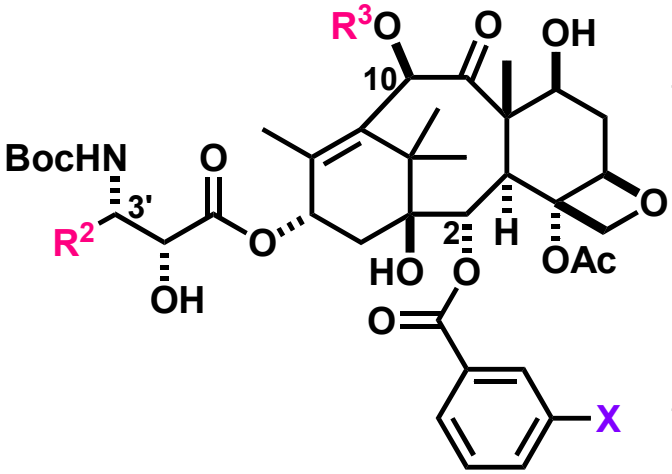
X = Me, F, Cl, N_3, OMe

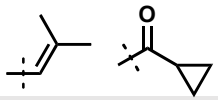
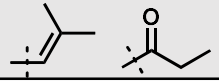
Synthesis of 3rd generation taxoids



1) Ojima, I.; Chen, J.; Sun, L.; Borella, C. P.; Wang, T.; Miller, M. L.; Lin, S. N.; Geng, X. D.; Kuznetsova, L. R.; Qu, C. X.; Gallager, D.; Zhao, X. R.; Zanardi, I.; Xia, S. J.; Horwitz, S. B.; Mallen-St Clair, J.; Guerriero, J. L.; Bar-Sagi, D.; Veith, J. M.; Pera, P.; Bernacki, R. J. *J. Med. Chem.* **2008**, *51*, 3203.

SAR of 3rd Generation Taxoids



cytotoxicity IC_{50} [nM][1)]	R^2	R^3	X	MCF7 (Pgp-)	NCI/ADR (Pgp+)	R/S[a]
taxol				1.7	300	176
docetaxel	Ph	H	H	1.0	235	235
SB-T-1214			H	0.20	2.1	10.5
SB-T-121303			OMe	0.36	0.43	0.92

[a] resistance factor = IC_{50}(NCI/ADR)/IC_{50}(MCF7)

3rd generation taxoids can circumvent Pgp-mediated MDR.

cytotoxicity IC_{50} [nM][2)]	HeLa (cervical)	HeLa (βIII)[a]	R/S[c]	1A9 (breast)	PTX10 (MT)[b]	R/S[c]
taxol	0.7	7.7	11	3.9	81.4	20.8
SB-T-1214	2.1	11.2	5.3	5.0	10.8	2.2
SB-T-121303	5.7	4.8	0.8	3.3	2.9	0.9

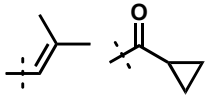
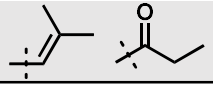
[a] transfected βIII tubulin [b] point mutation of β-tubulin [c] resistance factor = IC_{50}(drug resistance cell)/IC_{50}(WT)

3rd generation taxoids showed potency to taxol resistant cell due to β-tubulin diversification.

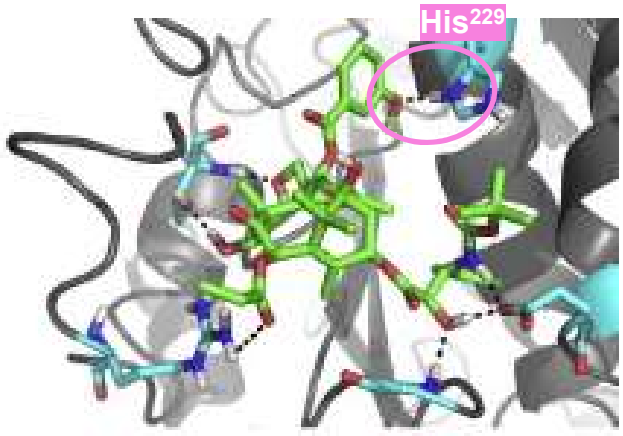
1) Ojima, I.; Chen, J.; Sun, L.; Borella, C. P.; Wang, T.; Miller, M. L.; Lin, S. N.; Geng, X. D.; Kuznetsova, L. R.; Qu, C. X.; Gallager, D.; Zhao, X. R.; Zanardi, I.; Xia, S. J.; Horwitz, S. B.; Mallen-St Clair, J.; Guerriero, J. L.; Bar-Sagi, D.; Veith, J. M.; Pera, P.; Bernacki, R. J. *J. Med. Chem.* **2008**, *51*, 3203. 2) Matesanz, R.; Trigili, C.; Rodriguez-Salarichs, J.; Zanardi, I.; Pera, B.; Nogales, A.; Fang, W. S.; Jimenez-Barbero, J.; Canales, A.; Barasoain, I.; Ojima, I.; Diaz, J. F. *Bioorg. Med. Chem.* **2014**, *22*, 5078.

Enhanced β-tubulin Affinity

binding parameters with β-tubulin

	R^2	R^3	X	IC_{50} (MCF7, nM)	K_A ($\times 10^7$ M)	ΔG (35 ℃) (kJ/mol)	ΔH (kJ/mol)	ΔS (kJ/mol K^{-1})
taxol	Ph	Ac	H	1.7	1.43	-42.1	-51	-29
docetaxel	Ph	H	H	1.0	3.93	-44.8	-53	-26
SB-T-1214			H	0.20	8	-46.6	-32	47
SB-T-121303			OMe	0.36	478	-57.0	-31	87

Higher cytotoxicity of SB-T taxoids may be due to the enhanced the β-tubulin affinity.



blue: β-tubulin green: SB-T-121303

The increased β-tubulin affinity of SB-T-121303 is probably due to hydrogen bonding of OMe at meta position of C2 benzoyl with His^{229}.

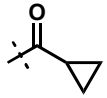
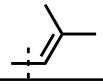
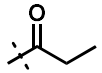
The effect of C3' and C10-OH modification on the β-tubulin affinity was yet rationalized.

Considering the significant differences of ΔH and ΔS, another interaction mode may be involved.

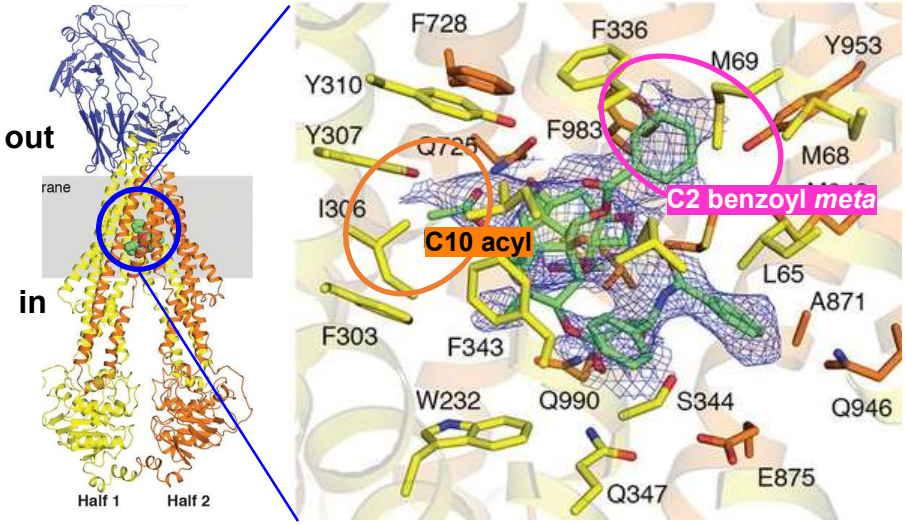
1) Matesanz, R.; Trigili, C.; Rodriguez-Salarichs, J.; Zanardi, I.; Pera, B.; Nogales, A.; Fang, W. S.; Jimenez-Barbero, J.; Canales, A.; Barasoain, I.; Ojima, I.; Diaz, J. F. *Bioorg. Med. Chem.* **2014**, *22*, 5078.

Enhanced Cytotoxicity against MDR Cells

multi drug resistant (MDR) cells: Pgp (efflux hydrophobic compounds) was overexpressed.

	R^2	R^3	X	R/S[a]
docetaxel	Ph	H	H	235
SB-T-1214			H	10.5
SB-T-121303			OMe	0.92

[a] resistance factor = IC_{50}(NCI/ADR)/IC_{50}(MCF7)



green: taxol, yellow: Pgp

(Cryo-EM (3.7 Å))

Pgp affinity may be decreased due to reduction of the hydrophobicity[1)].

Affinity with Pgp of 2nd and 3rd generation may be decreased by steric or electronic repulsion at C10 acyl chain and C2 benzoyl *meta*-substitution.

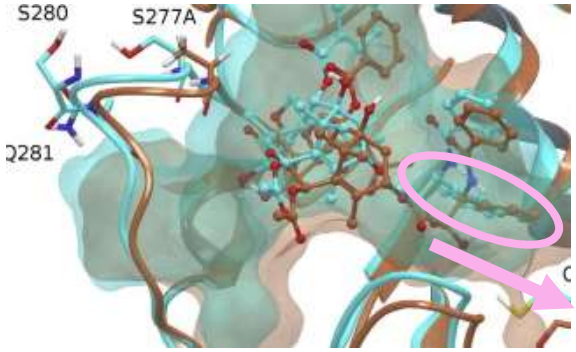
C10 acyl chain: Tyr^{307} or Ile^{306}
C2 benzoyl *meta*-substitution: Met^{69}, Tyr^{953}…

1) Matesanz, R.; Trigili, C.; Rodriguez-Salarichs, J.; Zanardi, I.; Pera, B.; Nogales, A.; Fang, W. S.; Jimenez-Barbero, J.; Canales, A.; Barasoain, I.; Ojima, I.; Diaz, J. F. *Bioorg. Med. Chem.* **2014**, *22*, 5078. 2) Alam, A.; Kowal, J.; Broude, E.; Roninson, I.; Locher, K. *Science* **2019**, *363*, 753.

Enhanced Cytotoxicity against β-tubulin mutant Cells

1. βIII overexpression

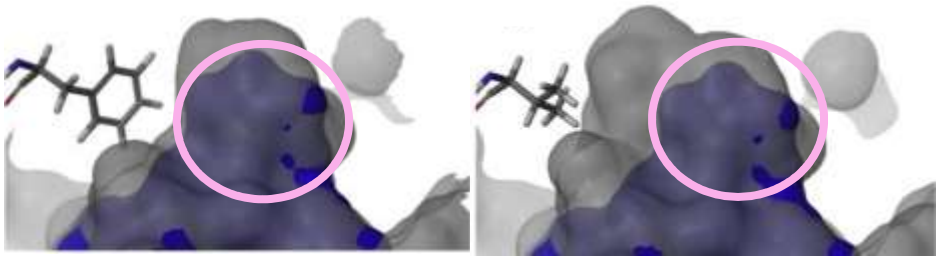
βIII… Ser^{277} to Ala^{277}
lost of H-bonding → larger pocket



blue: βI-taxol, brown: βIII-taxol

2. β-tubulin mutation

PTX10… Phe^{272} to Val^{272}
smaller residue → larger pocket



blue: taxol occupied volume, grey: binding volume

C3' phenyl became further from the pocket, which decreased the affinity.

cytotoxicity IC_{50} [nM][1)]	R^2	R/S (βIII)[a]	R/S (mutation)[a]
taxol	Ph	11	20.8
SB-T-1102		2.5	0.7
SB-T-1214		5.3	2.2

[a] resistance factor = IC_{50}(βIII transfected HeLa)/IC_{50}(HeLa-WT)
[b] resistance factor = IC_{50}(PTX10)/IC_{50}(1A9)

SB-T toxoids bearing larger entropic freedom of the side chain increased the affinity.

1) Matesanz, R.; Trigili, C.; Rodriguez-Salarichs, J.; Zanardi, I.; Pera, B.; Nogales, A.; Fang, W. S.; Jimenez-Barbero, J.; Canales, A.; Barasoain, I.; Ojima, I.; Diaz, J. F. *Bioorg. Med. Chem.* **2014**, *22*, 5078.

Contents

1. Introduction

2. Development 2nd and 3rd Generation Taxoids

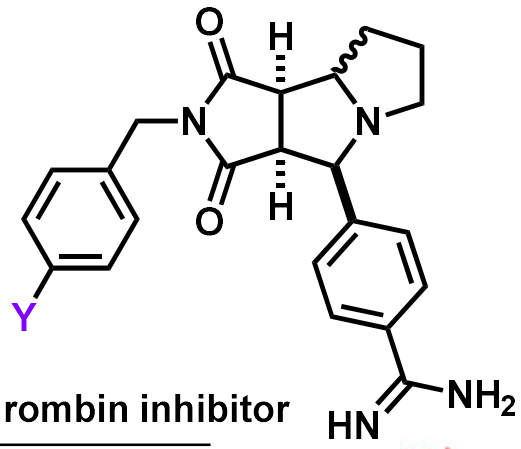
3. Fluorine Containing Taxoids

(*Bioorg. Chem.* **2022**, *119*, 105578)

Effect of Fluorine on Medicinal Chemistry

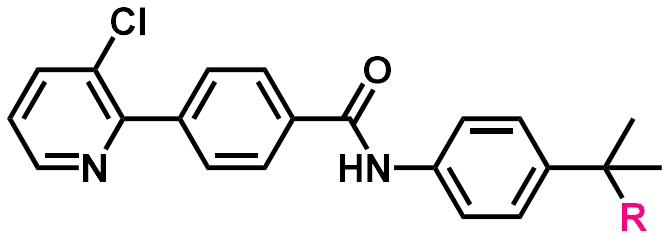
Fluorine introduction to compounds:

- **potency ↑, pK_a ↓, permeability ↑, clearance ↓**
- **conformational constraint**
- **positron emission tomography (PET, [^{18}F])**



thrombin inhibitor

Y	K_i (μM)
H	0.27
F	0.057



TRPV1 antagonist

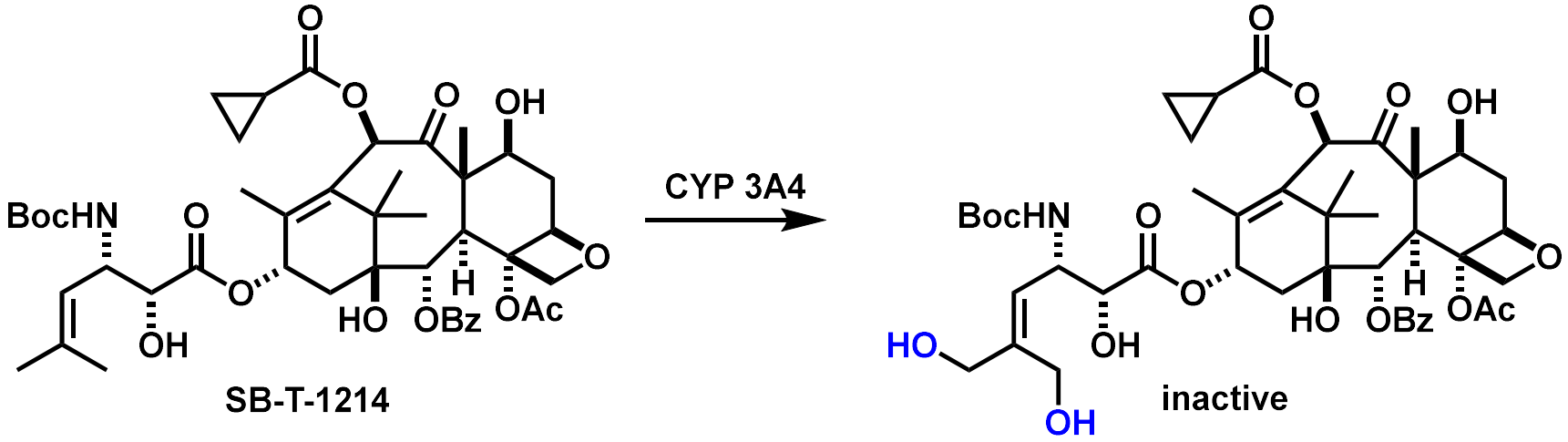
R	IC_{50} (nM)	HLM Cl_{int} (mL/min/kg)
Me	37	168
CF_3	42	46

Introduction of fluorine containing group improve drug utility by enhanced properties.

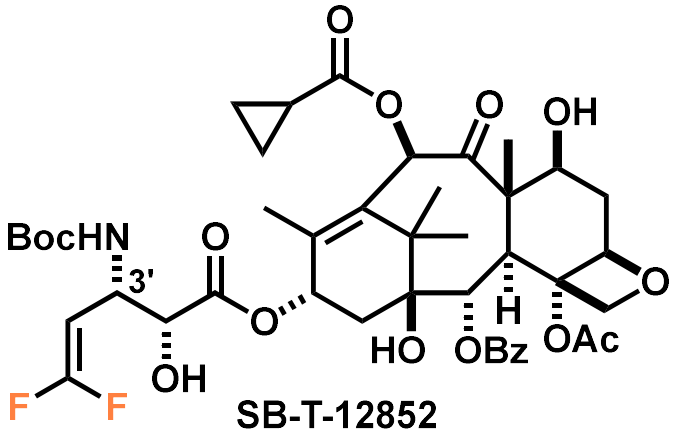
1) Cillis, P. E.; Eastman, J. K.; Hill, D. M.; Donnelly, J. D.; meanwell, A. N. *J. Med. Chem.* **2015**, *58*, 8315.

3’-Difluorovinyl (DFV) taxoids

2nd generation taxoid possessing 3’-isoprene were metabolized and loses the activity.[1)]



SB-T-1214



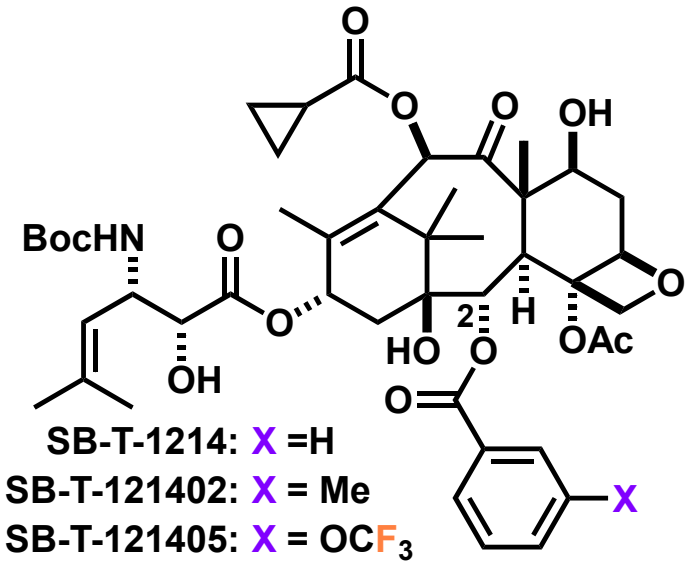
SB-T-12852

cytotoxicity IC_{50} [nM][2)]	MCF7 (breast)	NCI/ADR (ovarian, MDR)	R/S[a]
taxol	1.2	300	250
SB-T-1214	0.17	4.0	23.5
SB-T-12852	0.12	6.03	50.3

Fluorine substitution of vinyl position had no significant loss of the cytotoxicity.[2)]

1) Vaclavikov, R.; Soucek, P.; Svobodova, L.; Anzenbacher, P.; Simek, P.; Guengerich, FP, Gut, I. *Drug. Metab. Dispos.* **2004**, *32*, 666. 2) Kuznetsova, L.; Sun, L.; Chen, J.; Zhao, X. R.; Seitz, J.; Das, M.; Li, Y.; Veith, J. M.; Pera, P.; Bernacki, R. J.; Xia, S. J.; Horwitz, S. B.; Ojima, I. *J. Fluorine Chem.* **2012**, *143*, 177

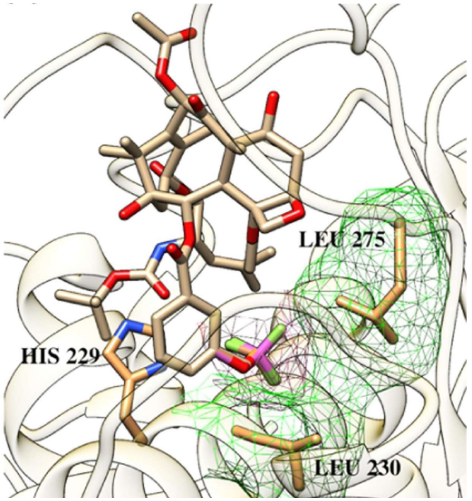
OCF_3 or OCF_2H Containing 3rd Generation Taxoids



SB-T-1214: X =H
SB-T-121402: X = Me
SB-T-121405: X = OCF_3
SB-T-121406: X = OCF_2H

cytotoxicity IC_{50} [nM][2)]	MCF7 (breast)	NCI/ADR (ovarian, MDR)
taxol	0.9	130
SB-T-1214	0.74	0.88
SB-T-121402	0.56	0.59
SB-T-121405	0.56	0.42
SB-T-121406	0.37	0.36

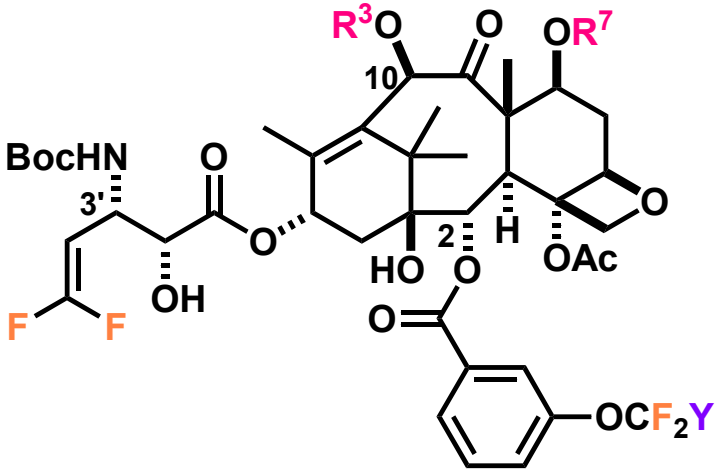
OCF_3 or OCF_2H substitution at *meta*-position efficiently enhancing the cytotoxicity.



The enhanced activity may be due to higher affinity with β-tubulin by van der Waals interaction of OCF_3 and OCF_2H with hydrophobic residue Leu^{230} and Leu^{275}.

1) Wang, C.; Wang, X.; Sun, Y.; Taouil, K. A.; Yan, S.; Botchkina, I. G.; Ojima, I. *Bioorg. Chem.* **2020,** *95*, 103523.

Multiple Fluorine Containing Taxoids[1)]



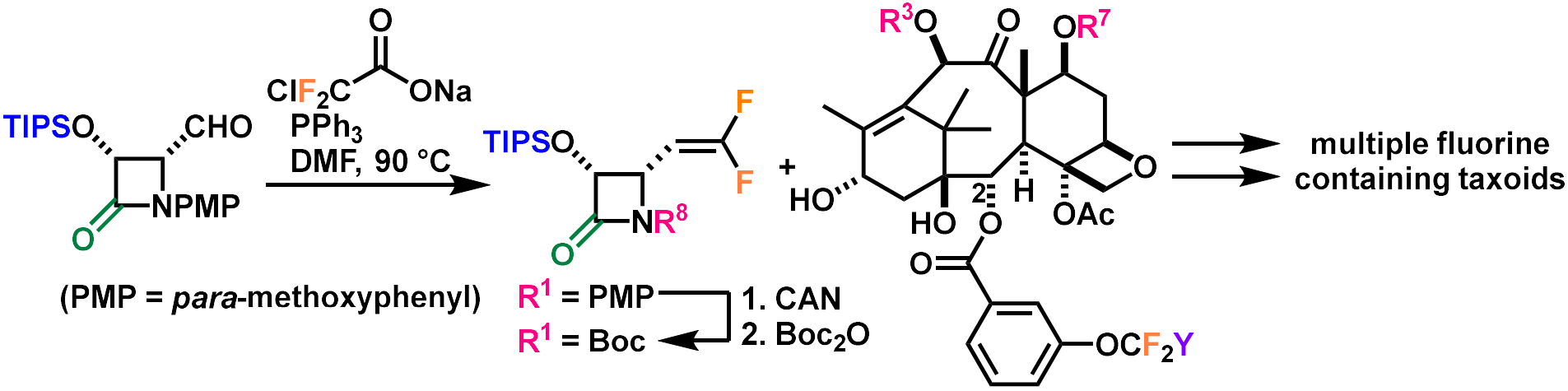
multiple fluorine containing taxoids:

R^3 = Acyl, R^7 = H or Me, Y = H or F

Hybridization of

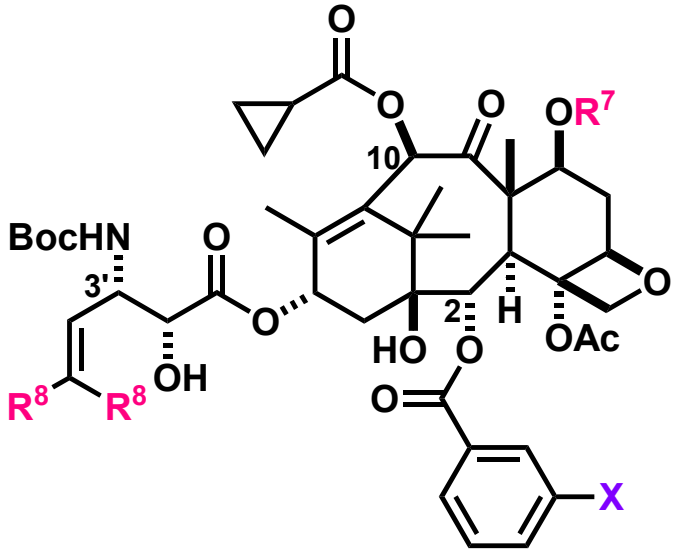
- **3'-Difluorovinyl (DFV)**
- **OCF_2Y at *meta*-position of C2 benzoyl**

Synthesis of multiple fluorine containing taxoids



1) Wang, C.; Chen, L.; Sun, Y.; Guo, W.; Taouil, K. A.; Ojima, I. *Bioorg. Chem.* **2022,** *119*, 105578.

Cytotoxicity of Fluorine Containing Taxoids (1)



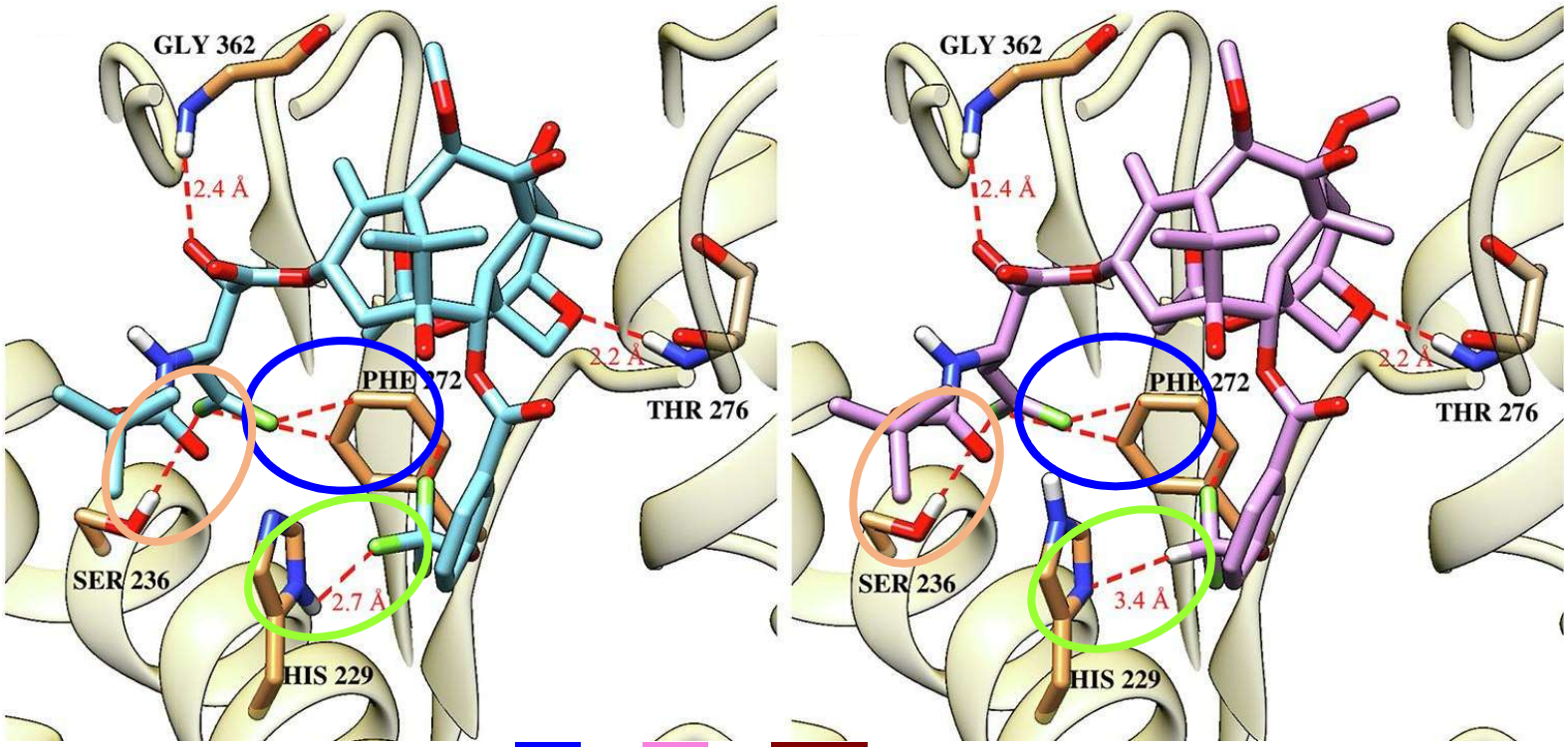
	R^7	R^8	X
SB-T-1214	H	Me	H
SB-T-12852	H	F	H
SB-T-121405	H	Me	OCF_3
SB-T-121406	H	Me	OCF_2H
A	H	F	OCF_3
B	Me	F	OCF_2H

cytotoxicity IC_{50}[nM][2)]	MCF-7 (breast)	PANC-1 (pancreatic)	NCI/ADR (ovarian, MDR)	DLD-1 (colon, MDR)	LCC6-MDR (breast, MDR)
SB-T-1214	0.20	0.33	1.2	4.00	2.59
SB-T-12852	0.14	0.52	3.45	-	-
SB-T-121405	0.15	-	0.75	2.98	1.71
SB-T-121406	0.10	-	0.97	3.65	1.46
A	0.12	0.34	-	0.56	0.565
B	0.08	0.43	-	0.09	0.248

Hybridization of DFV and OCF_2Y at *meta*-position of C2 benzoyl significantly enhanced the cytotoxicity against normal cancer cell and several MDR cells.

1) Wang, C.; Chen, L.; Sun, Y.; Guo, W.; Taouil, K. A.; Ojima, I. *Bioorg. Chem.* **2022,** *119*, 105578.

Molecular Docking Analysis



blue: A, pink: B, brown: β-tubulin

Higher affinity with β-tubulin was suggested by

- **Attractive interaction of fluorine atoms (DFV and OCF_2Y) with aromatic π-system of Phe^{272}**
- **Attractive interaction of fluorine of DFV with Ser^{236}**
- **Hydrogen bonding with His^{229} (OCF_3-HN, OCF_2H-N)**

  slightly lower potency of OCF_3: necessity of imidazole tautomerization

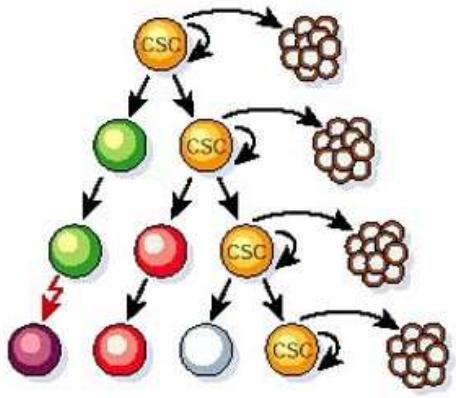
1) Wang, C.; Chen, L.; Sun, Y.; Guo, W.; Taouil, K. A.; Ojima, I. *Bioorg. Chem.* **2022,** *119*, 105578.

Cancer Stem Cell[1)]

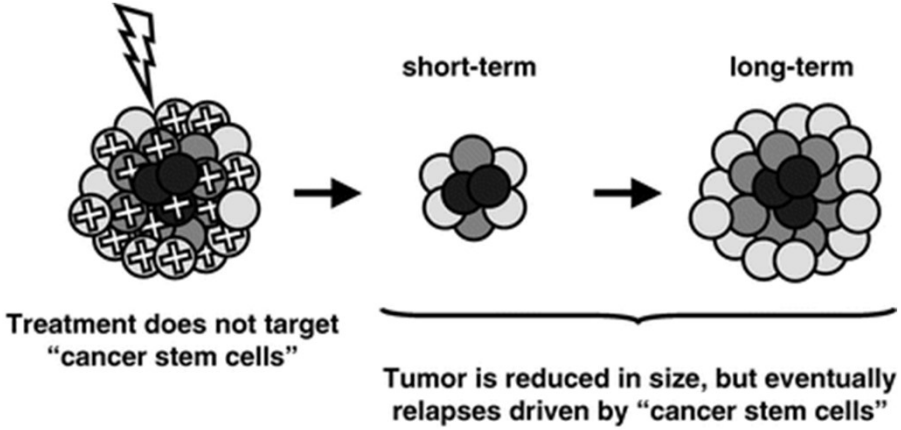
Cancer Stem Cell (CSC):

cancer cell with self-replication ability and pluripotent ability

- **induce a variety of proliferating,**
  but progressively differentiating tumor cells



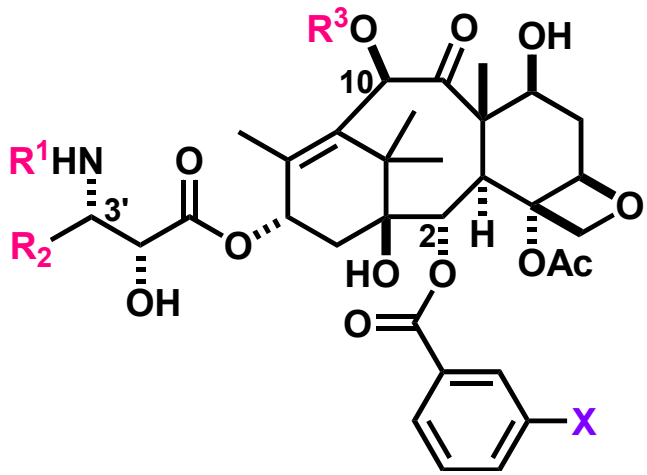
- **responsible for tumor maintenance, metastasis, resistance to treatment and recurrenc**

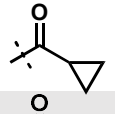
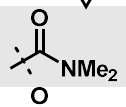
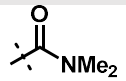


Recently, development of CSCs targeting drugs have become important.

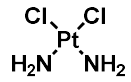
1) (a) Reya, T.; Morrison, S. J.; Clarke, M. F.; Weissman, I. L. *Nature* **2001**, *414*, 105. (b) Dalerba, P.; Cho, R. W.; Clarke, M. F. *Annu. Rev. Med.* **2007**, *58*, 267.

Cytotoxicity against CSCs (1)

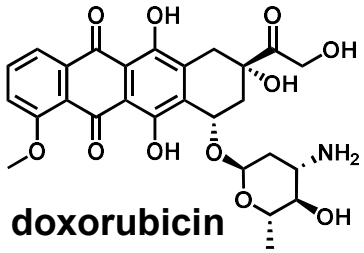


cytotoxicity IC_{50} [nM]	R^1	R^2	R^3	X	HCT-116 (CD133++)[a]
taxol	Bz	Ph	Ac	H	33.8
SB-T-1214	Boc			H	0.28
SB-T-12854	Boc			H	0.14
SB-T-121602	Boc			Me	0.24
cisplatin					4540
doxorubicin					78
methotrexate					32.7

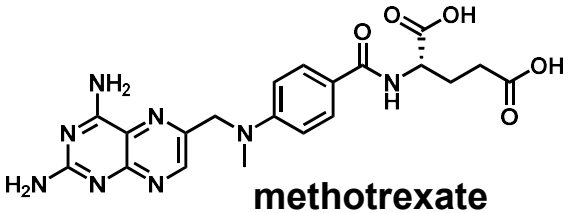
[a] CSC-enriched human colon cancer cell
CD133: highly expressed in early cell population



cisplatin



doxorubicin

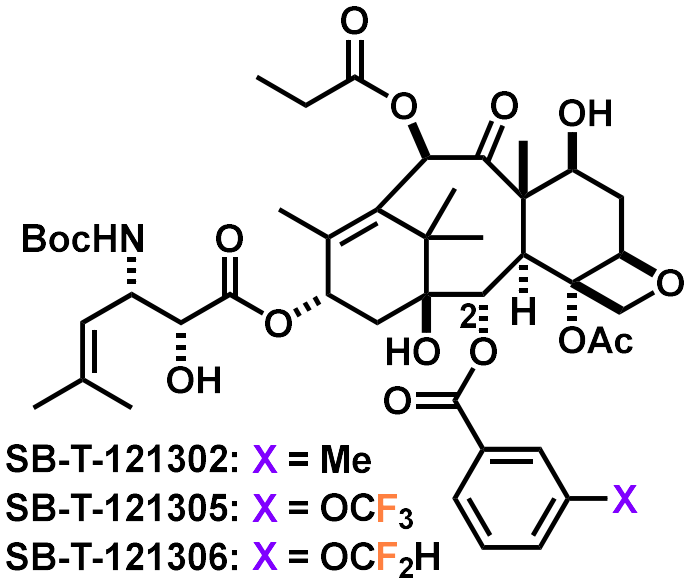


methotrexate

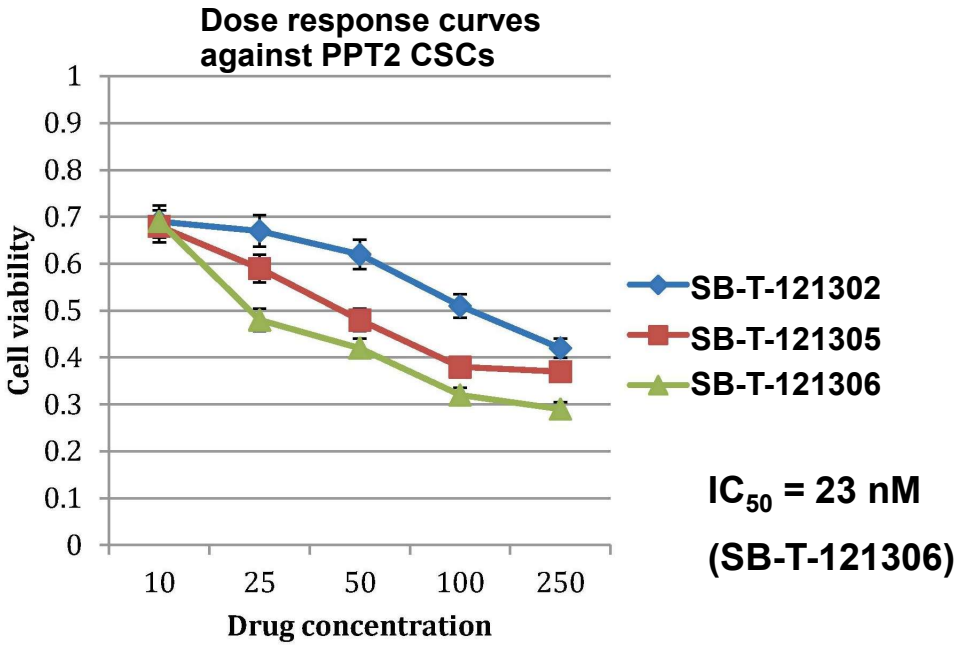
- 2nd and 3rd toxoids showed much higher cytotoxicity against highly drug resistant CSCs than conventional anticancer drugs.
- SB-T-12854 bearing DFV showed the highest activity, which suggested the advantage of fluorine substitution of vinyl position.

1) Zuniga, E. S. Ph.D. dissertation; Stony Brook University, 2012.

Cytotoxicity against CSCs (2)



SB-T-121302: X = Me
SB-T-121305: X = OCF_3
SB-T-121306: X = OCF_2H



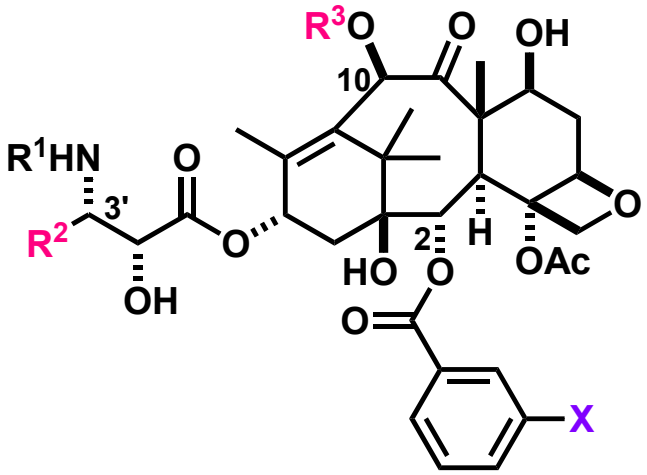
IC_{50} = 23 nM
(SB-T-121306)

Taxoids bearing OCF_3 or OCF_2H at *meta*-position of C2 benzoyl showed more potent cytotoxicity against PPT2 cancer stem cells than that bearing Me qroup.

Cytotoxicity against CSCs of multiple fluorine containing taxoids will be examined for the further investigation and development of anti CSCs taxoids.

1) Wang, C.; Chen, L.; Sun, Y.; Guo, W.; Taouil, K. A.; Ojima, I. *Bioorg. Chem.* **2022,** *119*, 105578.

Summary and Perspective



2nd and 3rd generation toxoids: modification of C3', C10, C2

1. improved anticancer activity (C3`, C2)

β-tubulin affinity ↑

2. enhanced anti resistant cancer cells (C10, C2)

Pgp affinity ↓, β-tubulin isotype affinity ↑

3. enhanced cytotoxicity against cancer stem cells

introducing fluorine may be more effective

Development of 2nd and 3rd generation toxoids dramatically enhanced the utility of taxol as the seed for anticancer agent.

Further study for

- **analysis of detailed mechanisms of anti CSCs activity**
- **direct suppression of undesirable side effects**

should be developed.