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SYNTHESIS OF TAXOIDS 5. SYNTHESIS AND EVALUATION OF NOVEL WATER-SOLUBLE PRODRUGS OF A 3'-DESPHENYL-3'-CYCLOPROPYL ANALOGUE OF DOCETAXEL

Tetsuo Yamaguchi, Naoyuki Harada, Kunihiko Ozaki, Hiroaki Arakawa, Kouji Oda, Noriyuki Nakanishi, Kenji Tsujihara, and Tomiki Hashiyama*

Medicinal Chemistry Research Laboratories, Tanabe Seiyaku Co., Ltd. 2-2-50, Kawagishi, Toda-shi, Saitama 335-8505, Japan

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Abstract: A novel 3'-desphenyl-3'-cyclopropyl analogue of docetaxel was synthesized from 10-deacetyl-baccatin III. The cytotoxicity of the new taxoid was evaluated against several human tumor cell lines, and it had ca. 20 times stronger activity against human colon cancer cell lines (WiDr and Colon 320) than that of docetaxel. This taxoid was converted to its water-soluble prodrugs that have 2'-substituted amino acid derivatives with spacer. The prodrugs had good solubility in saline and showed more potent antitumor activity against B16 melanoma in mice than that of docetaxel.

Introduction

A diterpenoid anticancer drug, paclitaxel **1**,[1] and its semisynthetic analogue, docetaxel **2**[2] that exhibits more potent antitumor activity than that of **1** in experimental models, have recently become indispensable drugs in clinics.[3] This is due to their potent antitumor activities especially against solid tumors, and unique mechanisms of action. However, these drugs have a number of undesirable side effects and are inactive against certain tumor types. And their water-insolubility hampers their clinical application. Because of this, these drugs should be co-injected with a detergent, Cremophor EL or Tween 80, which induces adverse effects such as hypersensitivity reactions. Due to attenuate the side effects, complicating injection is inescapable for clinics.[3a,4]

To overcome these problems, we set up two criteria. First one is finding a new taxoid which has a promising antitumor activity. Previously, a number of taxoids that have modified C-13 side chains,[5] were prepared and evaluated. Among them some 3'-desphenyl-3'-aryl[5b,5C] and alkyl[6] analogues have same or more potent antitumor activity than that of **2**. Herein, we envisaged that a cyclopropyl group which has not only alkyl function but also sp^2 character, would alternate the 3'-phenyl group. Then, we synthesized and evaluated a 3'-desphenyl-3'-cyclopropyl analogue **3** as a novel new lead compound (Figure 1).

Next, we aimed at producing water-soluble prodrugs of **3** with the primary goal of eliminating the toxic effects caused by detergents, and the secondary goal of modulating the pharmacology and toxicology of **3**.[7]

E-mail: tomiki@tanabe.co.jp Fax: +81-48-433-2610

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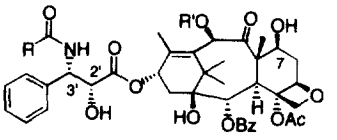
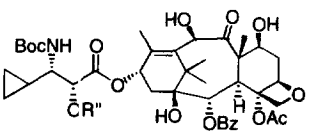
Figure 1



1 R = Ph, R' = Ac (paclitaxel)
2 R = *t*-BuO, R' = H (docetaxel)



3 R" = H
4 R" = H-A.A.-O • MsOH

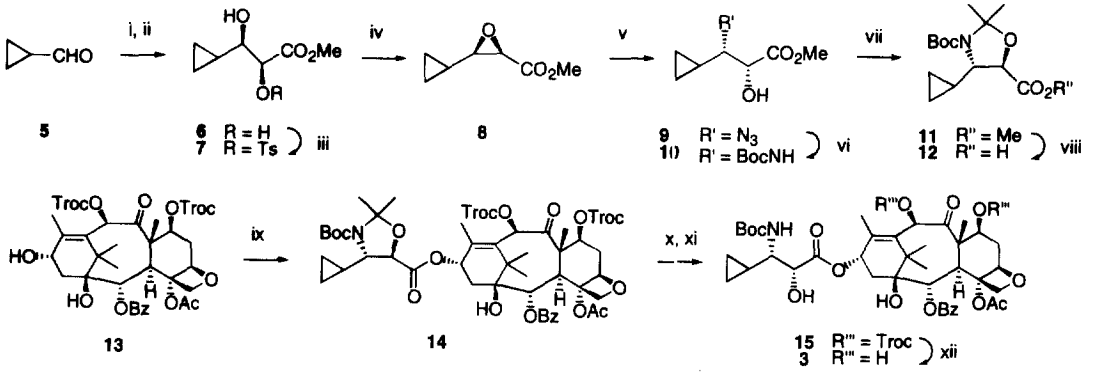
H-A.A.-OH = amino acid

To solve the problem of low water-solubility, several research groups have recently reported the synthesis and evaluation of water-soluble prodrugs of paclitaxel having the functionalized hydroxyl group at 2' and/or 7-positions.[8] Deutsh *et al.* have reported the synthesis of 2'-paclitaxel esters with amino acid moieties.[8a] Although a few of them exhibited potent antitumor activity, they were quite reactive and underwent chemical hydrolysis readily. We speculated that the poor chemical stability was caused by steric repulsion of bulky 2'-amino acid derivatives and 3'-position and neighboring effect of amino group. To avoid these undesired effects, we designed 2'-substituted water-soluble prodrugs **4**, which have amino acid derivatives with a glycolate spacer. Here, we wish to report the preparation of water-soluble taxoids **4** and their promising antitumor activity.

Chemistry

The synthesis of the 3'-desphenyl-3'-cyclopropyl analogue **3** is shown in Scheme 1. The α,β-unsaturated ester from **5** was subjected to the asymmetric dihydroxylation (AD) afforded the enantiomerically pure (2*S*,3*R*)-diol **6**. The diol **6** was converted to the monotosylate **7**, which was treated with K_2CO_3 to afford

Scheme 1

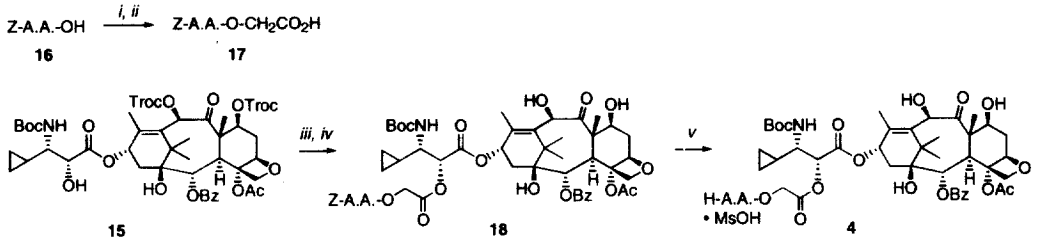


Reagents and conditions: (i) $Ph_3P{=}CHCO_2Me$, toluene, 55 °C, 18 h, 65%; (ii) AD-mix-ß, *t*-BuOH-H_2O, rt, 1 d, 48%; (iii) TsCl, NEt_3, CH_2Cl_2, 0 °C, 42 h, 81%; (iv) K_2CO_3, H_2O, CH_3CN, 50 °C, 1 d, 100%; (v) NaN_3, HCO_2Me, H_2O-MeOH, 50 °C, 14 h, 81%; (vi) Pd-C, H_2, Boc_2O, AcOEt, rt, 2.5 h, 96%; (vii) $CH_2{=}C(OMe)Me$, PPTS, toluene, 80 °C, 1.5 h, 96%; (viii) LiOH, H_2O-MeOH, rt, 1 h, 100%; (ix) **12** (1.5 eq), DCC, DMAP, toluene, 80 °C, 1.5 h, 100%; (x) HCO_2H, rt, 2 h, 97%; (xi) Boc_2O, $KHCO_3$, THF, rt, 3 h, 81%; (xii) Zn, AcOH, MeOH, 60 °C, 0.5 h, 85%.

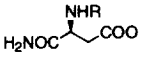
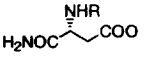
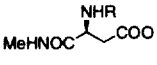
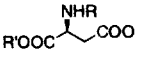
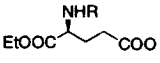
the *cis*-glycidic ester **8**. The reaction of **8** with sodium azide afforded **9**, which was subjected to the catalytic hydrogenation in the presence of Boc_2O to give *N-tert*-butoxycarbonyl-3-cyclopropylisoserin **10**. Cyclic protection of **10**[9] was carried out to give the oxazolidine **11**, which was saponified to yield the acid **12**. Esterification of baccatin III derivative **13**[9] using the acid **12** with DCC gave **14**. Deprotection of the oxazolidine-type protection followed by *N*-acylation afforded **15**. The deprotection of **15** with Zn gave **3**.

Aspartic acid derivatives **16a-d** and glutamic acid derivative **16e** were treated with *tert*-butyl bromoacetate[10] followed by deprotection to give the water-soluble auxiliaries **17** as shown in Table 1. The protected 3'-desphenyl-3'-cyclopropyl analogue **15** reacted with **17** gave the 2'-esters of **15**, followed by reductive deprotection of 2,2,2-trichloroethoxycarbonyl (Troc) groups gave the compounds **18**. The final catalytic hydrogenation in the presence of MsOH gave the water-soluble taxoids **4**.

Table 1: Synthesis of water-soluble taxoids **4**.



Reagents and conditions: (i) $BrCH_2CO_2t$-Bu, K_2CO_3, acetone, reflux; (ii) HCO_2H, rt; (iii) **17**, DCC, DMAP, THF, rt; (iv) Zn, AcOH, MeOH, 60 °C; (v) H_2, Pd-C, MsOH, THF.

R-A.A.-O	product: **17** yield (R = Z) (%)	product: **18** yield (R = Z) (%)	product: **4** yield (R = H) (%)	solubility of **4** in saline (mg/mL)
	**17a** 86	**18a** 55	**4a** 74	5
	**17b** 84	**18b** 58	**4b** 76	50
	**17c** 82	**18c** 72	**4c** 58	10
	**17d** 78 (R' = Bn)	**18d** 46 (R' = Bn)	**4d** 66 (R' = H)	1
	**17e** 70	**18e** 67	**4e** 73	10

The salt of 2'-esters **4** had the greatly improved solubility in saline (1~50 mg/mL). Furthermore, **4** were found to be chemically stable in saline. According to our expectation, the glycolate spacer was found to be a effective moiety to make the prodrugs with amino acids at 2'-OH stable.[11]

Evaluation and Discussion

The cytotoxicity of 3'-desphenyl-3'-cyclopropyl analogue **3** of docetaxel was evaluated against several human xenografts as shown in Table 2. The analogue **3** showed ca. 20 times stronger activity against human colon cancer cell lines (WiDr and Colon 320) than that of docetaxel **2**.

Table 2: Cytotoxic activities against human xenografts of 3'-desphenyl-3'-cyclopropyl analogue **3** and docetaxel **2**.

	IC_{50} (nM)*					
compound	SK-LU-1 (lung)	MKN1 (stomach)	OVCAR-3 (ovarian)	SK-BR-3 (breast	WiDr (colon)	Colon 320 (colon)
3	1.0	1.2	0.52	1.1	0.027	0.21
docetaxel **2**	1.8	1.7	1.2	4.7	0.43	4.8
ratio**	1.8	1.4	2.3	4.3	16	23

*Cytotoxicity was determined by the MTT assay. The incubation time was 72 h and IC_{50} values are expressed as the concentration which causes a 50% decrease in cell viability. **IC_{50} (**2**) / IC_{50} (**3**).

Next, **3** and water-soluble taxoids **4** were evaluated for antitumor activity by intravenous administration against B16 melanoma (implanted subcutaneously) in mice (Table 3). Docetaxel **2**, which is regarded as most powerful antitumor taxoid *in vivo*, was included for comparison.

Table 3: Antitumor activity of **3** and **4** against B16 melanoma. *

compd.	optimal dose of drug (mg/kg/day)	ILS** (%)	body weight change on day 10 (%)
4a	12.5	227	-3.2
4b	6.3	127 ***	-11.1
4c	6.3	185	-0.5
4d	25	212 ***	3.3
4e	12.5	213	-2.9
3	6.3	187	-7.5
docetaxel (**2**)	20	149	-13.0

* B16 melanoma cells were inoculated subcutaneously in 5 mice, and each compound was administered iv on days 1 to 5. ** Increase in life span of mice when treated at optimal dose.
ILS(%) = (mean survival time of treated group (except cured mouse***) / that of control group - 1) x 100.
*** One mouse survived on day 90, and the tumor was undetectable.

We found that **3** showed more potent *in vivo* antitumor activity than that of **2**, which was determined by the comparison of increase in life span (ILS) of B16 melanoma-bearing mice. Futhermore, several compounds **4** showed antitumor activity superior to **2** and **3**. Especially, **4a**, which showed the largest ILS value, had excellent antitumor activity at wide range of dose (the ILS value of **4a** at dose 6.3 mg/mL/day was 165%). Judging from the body weight change **4a**,**4d**,**4e** appeared to be less toxic than **3**, an active metabolite of **4**.

This was probably due to modulating the pharmacology by water-solubility. To our knowledge, **4** are effective water-soluble prodrugs of taxoid which are compatible with strong antitumor activity *in vivo* and stablity in water. Potent antitumor activities in experimental models using human tumor xenografts in athymic nude mice and good results of the pharmacokinetic studies of **4a** will be submitted soon.

In conclusion, we synthesized water-soluble prodrugs **4** of 3'-desphenyl-3'-cyclopropyl analogue **3**. These compounds had good solubility and stability in saline. Moreover, most of them showed potent antitumor activity against B16 melanoma, compared with **3** and docetaxel **2**.

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References and Notes

1) Wani, M. C.; Taylor, H. L.; Wall, M. E.; Coggon, P.; McPhail, A. T. *J. Am. Chem. Soc.* **1971**, *93*, 2325.

2) Gueritte-Voegelein, F.; Guenard, D.; Lavelle, F.; Le Goff, M. -T.; Mangatal, L.; Potier, P. *J. Med. Chem.* **1991**, *34*, 992.

3) (a) Rowinsky, E. K.; Donehower, R. C. *New Engl. J. Med.* **1995**, *332*, 1004; (b) Georg, G. I.; Chen, T. T.; Ojima, I.; Vyas, D. M. *ACS Symposium Series 583*; American Chemical Society: Washington, DC, 1995; 31.

4) (a) Slichenmyer, W. J.; Hoff, D. D. V. *J. Clin. Pharmacol.* **1990**, *30*, 770; (b) Dorr, R. T. *Ann. Pharmacother.* **1994**, *28*, S11; (c) Rose, W. C.; Clark, J. L.; Lee, F. Y. F.; Casazza, A. M. *Cancer Chemother. Pharmacol.* **1997**, *39*, 486.

5) For a review, see: (a) Nicolaou, K. C.; Dai, W. -M.; Guy, R. K. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 15; (b) Georg, G. I.; Ali, S. M.; Zygmunt, J.; Jayasinghe, L. R. *Exp. Opin. Ther. Patents* **1994**, *4*, 109; (c) Hepperle, M.; Georg, G. I. *Drugs of the Future*, **1994**, *19*, 573. (d) For our previous results: Yamaguchi, T.; Harada, N.; Ozaki, K.; Hayashi, M.; Arakawa, H.; Hashiyama, T. *Tetrahedron* **1999**, *55*, 1005 and references cited therein.

6) (a) 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; (b) 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; (c) Ali, S. M.; Hoemann, M. Z.; Aube, J.; Georg, G. I.; Mitscher, L. A. *J. Med. Chem.* **1997**, *40*, 236; (d) Ojima, I.; Kuduk, S. D.; Pera, P.; Veith, J. M.; Bernacki, R. J. *J. Med. Chem.* **1997**, *40*, 279 and references cited therein; (e) Substituted cyclopropyl analogues: Ojima, I.; Lin, S. *J. Org. Chem.* **1998**, *63*, 224.

7) We have already synthesized water-soluble prodrugs of 9-hydroxyellipticine having various amino acids, which have potent antitumor activity and improved pharmacokinetics and tissue distribution. Harada, N.; Ozaki, K.; Oda, K.; Nakanishi, N.; Ohashi, M.; Hashiyama, T.; Tsujihara, K. *Chem. Pharm. Bull.* **1997**, *45*, 1156.

8) (a) Deutsh, H. M.; Glinski, J. A.; Hernandez, M.; Haugwitz, R. D.; Narayanan, V. L.; Suffness, M.; Zalkow, L. H. *J. Med. Chem.* **1989**, *32*, 788; (b) Mathew, A. E.; Mejillano, M. R.; Nath, J. P.; Himes, R. H.; Stella, V. J. *J. Med. Chem.* **1992**, *35*, 145; (c) Greenwald, R. B.; Pendri, A.; Bolikal, D. *J. Org. Chem.* **1995**, *60*, 331; (d) Ueda, Y.; Matiskella, J. D.; Mikkilinent, A. B.; Farina, V.; Knipe, J. O.; Rose, W. C.; Casazza, A. M.; Vyas, D. M. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 247; (e) Golik, J.; Wong, H. S. L.; Chen, S. H.; Doyle, T. W.; Wright, J. J. K.; Knipe, J.; Rose, W. C.; Casazza, A. M.; Vyas, D. M. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1837; (f) Greenwald, R. B.; Gilbert, C. W.; Pendri, A.; Conover, C. D.; Xia, J.; Martinez, A. *J. Med. Chem.* **1996**, *39*, 424; (g) Takahashi, T.; Tsukamoto, H.; Yamada, H. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 113; (h) Bourzat, J. D.; Commerçon, A. PTC Patent Appl., WO 9323389-A1, 1993.

9) Commerçon, A.; Bezard, D.; Bernard, F.; Bourzat, J. D. *Tetrahedron Lett.* **1992**, *33*, 5185.

10) Lee, S. D.; Chan, T. H.; Kwon, K. S. *Tetrahedron Lett.* **1984**, *25*, 3399.

11) A solution of **4a** in saline was standed at room temperature for 5 h, >95% of **4a** was remained. In contrast, Deusch *et al.* Reported[8a] that all attempts to prepare basic prodrugs of paclitaxel with amino acid esters failed, because these compounds are quite unstable and readily revert to paclitaxel.