This article is dedicated to Professor Satoshi Ōmura in celebration of his 2015 Nobel Prize.

Note

Chemistry of Ecteinascidins. Part 5: An Additional Proof of Cytotoxicity Evaluation of Ecteinascidin 770 Derivatives

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Eleven 2'-N-acyl derivatives (**5a–k**) were prepared from ecteinascidin 770 (Et 770: **1b**) via known 18,6'-O-bisallyl-protected compound (**3**) in three steps. Their *in vitro* cytotoxicities were determined by measuring IC₅₀ values against human cell lines HCT116 and DU145. 5-Isoxazolecarboxylamide derivative (**5i**) and 4-methoxybenzoylamide derivative (**5k**) were found to be promising leads for further optimization.

Key words marine natural product; transformation; ecteinascidin; cytotoxicity; structure–activity relationship study

Ecteinascidin 743 (**1a**) is known by its international non-proprietary name “trabectedin” and is being marketed under the trade name “Yondelis®.”^{1–5} It was approved in September 2007 in the European Union for use in the treatment of advanced soft tissue sarcoma, and is currently being used in almost 80 countries and regions around the world, including the United States and Japan.⁶ However, despite its medicinal importance, there are very few structure–activity relationship (SAR)⁷ studies owing to the meager amount of the natural product and the low chemical diversity of the biosynthetically produced derivatives.^{8,9}

As a result of untiring efforts to establish the medicinal chemistry of marine-derived antitumor ecteinascidin 743 (Et 743: **1a**), we were able to prepare many ecteinascidin derivatives with an eye to improving cytotoxicity profiles.^{10–13} To extend the scope of SARs of these fascinating marine-derived natural products, we have focused our attention on the preparation of 2'-N-acyl derivatives in order to investigate their cytotoxicity profiles. In this paper, we report the preparation of eleven 2'-N-acyl derivatives from ecteinascidin 770 (Et 770: **1b**) via 18,6'-O-bisallyl-protected compound (**3**) in moderate yields, most of which exhibited slightly higher cytotoxicities than parent **1b** (Fig. 1).

We have reported that 2'-N-(3-quinolinecarbonyl)amide **2a**, 2'-N-4-fluorocinnamoyl derivative **2b**, and 2'-N-3-pyridine-cinnamyl derivative **2c** were extremely potent derivatives, possessing approximately 20- to 70-fold higher cytotoxicity to the HCT116 human colon carcinoma cell line than **1b**.¹³ Those results indicated that nitrogen-containing heterocyclic amide, fluorine-substituted aromatic amide, and cinnamyl amide analogs enhanced the cytotoxicity (Table 1).

Treatment of **3**¹³ with an acid chloride and a catalytic

amount of 4-dimethylaminopyridine (DMAP) in pyridine at 60°C for several hours gave 2'-N-acyl derivatives **4a–d** in 74.6–89.8% yields (Chart 1). The reaction of **4a–d** with tributyltin hydride, (Ph₃P)₂PdCl₂, and AcOH in tetrahydrofuran (THF) at 25°C for 1–4 h gave desired amides **5a–d** in 62.9–67.9% yields. The conversion of **3** into heterocyclic amide derivatives **5g–i** as well as 1-naphthalenecarbonyl amide derivative (**5j**) and 4-methoxybenzoyl amide derivative (**5k**) was achieved in moderate overall yields (45.2–72.6%). In contrast, attempts to prepare cinnamylamide derivatives having fluorine atoms in the aromatic ring **5e** and **f** via **4e** and **f** gave slightly low overall yields because several minor products were generated in the two reactions steps. The structures of the synthesized compounds were established on the basis of ¹H-NMR, ¹³C-NMR, MS, and infrared (IR) spectral data along with optical rotation values (see Supplementary Materials).

The above synthesized derivatives and **1b** were tested for *in vitro* cytotoxicity to two representative human solid carcinoma cell lines, HCT116 and DU145, using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Table 2 shows that the IC₅₀ values of eleven 2'-N-acyl derivatives **5a–k** along with **1b** were of nm order. In general, we found that the HCT116 cell line was more sensitive to the ecteinascidins than the DU145 cell line. Among the aromatic acyl derivatives, including cinnamylamide derivatives having fluorine atoms **5a–f**, derivatives **5a–e** exhibited slightly higher cytotoxicities to HCT116 and DU145 cell lines than **1b**. However, all compounds having fluorine atoms showed significantly lower cytotoxicities than known compound **2b**.

We then turned our attention to sulfur-containing heterocycles and isoxazole derivatives, such as **5g–i**, as well as 1-naph-

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Fig. 1. Structures of Ecteinascidin Marine Natural Product and Related Analogs

Table 1. Cytotoxicities of Et 770 (**1b**) and Its 2'-N-Acyl Analogues to Three Carcinoma Cell Lines (IC₅₀ μ M)

Entry	Compound	18,6'-O-Bisallyl derivatives of Et 770	Cytotoxicity		
			HCT116 ^{a)}	QG56 ^{a)}	DU145 ^{a)}
1	1b ^{b)}	(Ecteinascidin 770)	0.71	1.60	1.60
	1b ^{c)}		0.60	2.40	0.81
2	2a	3-Quinolincarbonyl	0.014	0.071	0.087
3	2b	4-Fluorocinnamoyl	0.010	0.12	0.041
4	2c	3-C ₅ H ₄ NCH=CHCO-	0.031	0.068	0.089

a) HCT116: human colon carcinoma; QG56: human lung carcinoma. DU145: human prostate carcinoma. b) For previous cytotoxicity data, see ref. 13. c) See ref. 12.

thalene amide derivative **5j** and 4-methoxyphenyl amide **5k**. 5-Isoxazole amide **5i** and 4-methoxyphenyl amide **5k** exhibited significantly higher cytotoxicity to both cell lines than **1b**. However, all the compounds showed lower cytotoxicities than the previously reported compounds **2a** and **c**.

In conclusion, we found that the introduction of acyl groups at the 2'-N position enhanced the cytotoxicity. 2'-N-Isioxazole amide derivative **5i** and 2'-N-(4-methoxyphenyl)amide derivative **5k** were the most potent derivatives, exhibiting approximately 4.9- to 6.1-fold increase in cytotoxicity to the HCT116 cell line, and 6.6- to 7.5-fold increase in cytotoxicity to the DU145 cell line, respectively, relative to **1b**.

Our findings offer new evidence that the 2'-N-acyl derivatives play a novel role in the antitumor activity of ecteinascidin analogs. Further studies are needed to completely understand the molecular basis of the extraordinary cytotoxicity profiles of ecteinascidins.

Experimental

¹H- and ¹³C-NMR spectra were obtained on JEOL JNM-AL 300 and JEOL-JNM-ECA 500 FT NMR spectrometers. Solvent signals served as the internal standard (CDCl₃: δ_H 7.26/ δ_C 77.0). IR spectra were recorded on a Shimadzu IR Affinity-1 Fourier Transform Infrared (FT-IR) spectrophotometer. High-resolution (HR)-MS were acquired from a JEOL JMS 700 mass spectrometer with a direct inlet system operating at 70 eV. Optical rotations were measured with a Horiba SEPA-200 polarimeter. Circular dichroism (CD) measurements

were conducted with a Jasco J-820 spectropolarimeter. Silica gel 60 (230-400 and 70-230 mesh) and Sephadex[®] LH-20 were used for column chromatography.

General Procedure for Preparation of 18,6'-O-Bisallyl-2'-N-acyl Derivatives of Et 770 (4a-k) Compound **3** (15.3 mg, 0.018 mmol) and DMAP (1.1 mg) were dissolved in pyridine (1.5 mL), and 15 equivolar quantity of an acid chloride was added at 0°C. The reaction mixture was heated at 60°C for 2 to 3 h. After the solvent was removed *in vacuo*, the residue was diluted with water (10 mL) and extracted with chloroform (15 mL×3). The combined extracts were washed with brine (15 mL), dried, and concentrated *in vacuo* to give a residue. The residue was purified by silica gel column chromatography using an appropriate eluent to give the corresponding purified products (**4a-k**).

General Procedure for Preparation of 2'-N-Acyl Derivatives of Et 770 (5) Tributyltin hydride (0.064 mL, 0.237 mmol) was added dropwise over 10 min to a vigorously stirred solution of **4a-k** (14.4 μ mol), (Ph₃P)₂PdCl₂ (6.0 mg, 8.6 μ mol), and AcOH (0.03 mL, 0.54 mmol) in THF (4 mL) at 25°C, and the reaction mixture was stirred at 25°C for 1–4 h. The reaction mixture was diluted with water (10 mL), made alkaline with 5% aqueous NaHCO₃, and extracted with chloroform (30 mL×3). The combined extracts were washed with 5% aqueous NaHCO₃, dried, and concentrated *in vacuo* to give a residue. The residue was purified by silica gel column chromatography using an appropriate eluent to give the corresponding purified products (**5a-k**).

Chart 1. Preparation of 2'-N-Acyl Derivatives of Et 770

Table 2. Cytotoxicities of 2'-N-Acyl Derivatives of **1b** to Two Carcinoma Cell Lines (IC₅₀ nM) along with Isolation Yields

Entry	Compound	2'-N-Acyl derivatives of Et 770	Isolation yield (%)		Cytotoxicity (5)	
			4	5	HCT116 ^{a)}	DUI45 ^{a)}
1	1b	(Ecteinascidin 770)	—	—	2.6	4.6
2	a	2-Fluoro-4-methylphenyl	74.6	62.9	1.8	4.5
3	b	4-Ethoxy-2,3-difluorophenyl	89.8	63.2	0.96	1.0
4	c	2,3,4-Trifluorophenyl	83.1	67.9	1.0	3.3
5	d	2,4,5-Trifluorophenyl	82.9	66.4	1.7	2.6
6	e	(2,3,4,5,6-Pentafluorophenyl)prop-2-ene	83.4	21.4	1.7	3.0
7	f	(4-Trifluoromethylphenyl)prop-2-ene	52.6	51.7	22.8	89.0
8	g	2-Thiophenyl	98.3	73.9	1.8	4.5
9	h	3-Thiophenyl	89.6	72.8	2.6	6.2
10	i	5-Isoxazolyl	97.6	52.6	0.42	0.61
11	j	1-Naphthyl	84.5	53.5	8.4	17.8
12	k	4-Methoxyphenyl	94.9	56.5	0.53	0.69

a) HCT116: human colon carcinoma; DUI45: human prostate carcinoma.

Cell Growth Inhibition Assay (IC₅₀) A single-cell suspension of each cell line (2×10³ cells/well) was added to the serially diluted test compounds in a microplate. The cells were then cultured for 4d. Cell growth was measured with a cell counting kit (DOJINDO, Kumamoto, Japan). IC₅₀ was expressed as the concentration at which cell growth was inhibited by 50% compared with the untreated control.

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Conflict of Interest The authors declare no conflict of

interest.

Supplementary Materials The online version of this article contains supplementary materials. ^1H -NMR, ^{13}C -NMR, IR, and MS spectral data, and optical rotation values of all compounds are available.

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