Ecteinascidin 743 (1a) is known by its international non-proprietary name “trabectedin” and is being marketed under the trade name “Yondelis®.” 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. However, despite its medicinal importance, there are very few structure–activity relationship (SAR) studies owing to the meager amount of the natural product and the low chemical diversity of the biosynthetically produced derivatives.

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. 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 known 18,6'-O-bisallyl-protected compound (3) in three steps. Their in vitro cytotoxicities were determined by measuring IC_{50} values against human cell lines HCT116 and DU145. 5-Isoxazolecarboxyamide 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

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 IC_{50} 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 fluoride 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 	extsuperscript{1}H-NMR, 	extsuperscript{13}C-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 IC_{50} 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 fluoride atoms 5a–f, derivatives 5a–e exhibited slightly higher cytotoxicities to HCT116 and DU145 cell lines than 1b. However, all compounds having fluoride 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-...
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 2c.

In conclusion, we found that the introduction of acyl groups at the 2′-N position enhanced the cytotoxicity. 2′-N-(4-methoxyphenyl) 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 ecteinascidins. 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; δH 7.26/ δC 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 70eV. 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 equimolar 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).

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

| Entry | Compound | 18,6′-O-Bisallyl derivatives of Et 770 | Cytotoxicity |
|-------|----------|-------------------------------------|-------------|
|       |          | (Ecteinascidin 770)                 | HCT116(a)  |
| 1     | 1b<sup>b</sup> | 0.71                                | 1.60        |
| 1     | 1b<sup>c</sup> | 0.60                                | 2.40        |
| 2     | 2a       | 0.014                               | 0.071       |
| 3     | 2b       | 0.010                               | 0.12        |
| 4     | 2c       | 0.031                               | 0.068       |

Table 1. Cytotoxicities of Et 770 (1b) and Its 2′-N-Acyl Analogues to Three Carcinoma Cell Lines (IC50 µM)

- 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.
Cell Growth Inhibition Assay (IC₅₀) A single-cell suspension of each cell line (2×10⁴ cells/well) was added to the serially diluted test compounds in a microplate. The cells were then cultured for 4 d. 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.

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

Table 2. Cytotoxicites 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 (IC₅₀ nM) |
|-------|----------|-------------------------------|---------------------|-----------------------|
|       |          |                               | HCT116       | DU145                 |
| 1     | 1b       | (Eteinasidin 770)             | —           | 2.6                  |
| 2     | a        | 2-Fluoro-4-methylphenyl       | 74.6        | 6.2                  |
| 3     | b        | 4-Ethoxy-2,3-difluorophenyl   | 89.8        | 63.2                 |
| 4     | c        | 2,3,4-Trifluorophenyl         | 83.1        | 67.9                 |
| 5     | d        | 2,4,5-Trifluorophenyl         | 82.9        | 66.4                 |
| 6     | e        | (2,3,4,5,6-Pentafluorophenyl)prop-2-ene | 83.4 | 21.4                 |
| 7     | f        | (4-Trifluoromethylphenyl)prop-2-ene | 52.6 | 51.7                 |
| 8     | g        | 2-Thiophenyl                 | 98.3        | 73.9                 |
| 9     | h        | 3-Thiophenyl                 | 89.6        | 72.8                 |
| 10    | i        | 5-Isoxazolyl                 | 97.6        | 52.6                 |
| 11    | j        | 1-Naphthyl                   | 84.5        | 53.5                 |
| 12    | k        | 4-Methoxyphenyl              | 94.9        | 56.5                 |

a) HCT116: human colon carcinoma; DU145: human prostate carcinoma.
interest.

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

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