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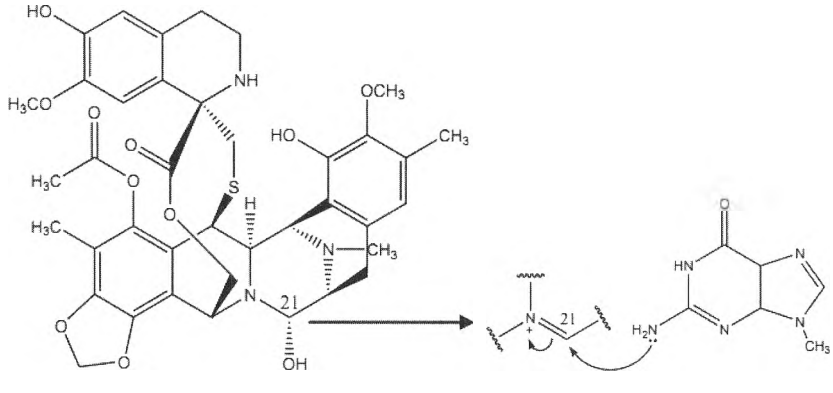
INTRODUCTION

Ecteinascidin 743 (Et 743, Trabectedin, Yondelis®, **Scheme 1**), a tris-tetrahydroisoquinoline alkaloid isolated from the Caribbean tunicate *Ecteinascidia turbinata*,[1)] has been approved by European Medicines Agency for using in the patients with soft tissue sarcoma.[2)] The unique chemical structure of Et 743 is composed of tetrahydroisoquinoline 3 subunits (A, B and C) and an α-carbinolamine functional group responsible to its anticancer activity. Et 743 was reported to induce the DNA sequence-selective alkylation of the guanine N_2 in the duplex DNA minor groove via iminium intermediate (**Scheme 1**).[3)] The NMR-based studies of the DNA and Et 743 interaction have shown that the A and B-subunits and α-carbinolamine are sequence binding recognition.[4)] Interestingly, the C-subunit, which is perpendicular to the A and B-subunits, protrudes from the duplex DNA binding sites and might be related to its DNA binding affinity.[5)] In addition, the α-carbinolamine is not only structurally crucial for the DNA-alkylation but also causing the compound unstabiliy. We have succeeded in isolating a large amount of the stable ecteinascidin 770 (Et 770, **1**) from the Thai tunicate *Ecteinascidia thurstoni* by pretreatment with potassium cyanide in buffer solution.[6)] In order to improve its biological activity, the chemical modification of **1** has been particularly focused at the C-subunit. The first preparation of the 2'-*N*-acyl derivatives of **1** was recently reported based on a three-step transformation including: a) 18,6'-*O*-bisallyl protection, b) 2'-*N*-acylation, and c) removing of the allyl protecting group.[7,8)] Most of them showed potent cytotoxicity on human solid tumor cell lines. In this presentation, preparation of 2'-*N*-2,3,4-trifluorobenzoyl derivative of **1** for future cytotoxicity evaluation will be reported.



Et 743 Iminium intermediate

Scheme 1 Et 743 and mechanism of action

MATERIALS AND METHODS

General experimental procedure Optical rotations were measured on a Perkin Elmer 341 polarimeter. The IR spectra were obtained on a Perkin Elmer Spectrum One FT-IR spectrometer. The ^{1}H and ^{13}C spectra were recorded at 500 and 125.65 MHz, respectively, on a JEOL JNM-LA-500 FT-NMR spectrometer and at 300 and 75 MHz, respectively, on a Bruker Avance DPX-300 FT-NMR spectrometer. The HR-FABMS spectra were recorded on a JMS-700 mass spectrometer, with a direct inlet system operating at 70 eV. CD was obtained on a Jasco J-715 spectropolarimeter. Column chromatography was

performed using Silica gel 60 (No. 1.09385, particle size 0.040-0.063 mm, Merck) for flash column chromatography, silica gel 60 (No. 1.07734, particle size 0.063-0.200 mm, Merck) for quick column chromatography and Sephadex LH-20 (Pharmacia Biotech AB) for gel filtration chromatography.

Extraction and isolation of Et 770 The tunicate *Ecteinascidia thurstoni* (65 kg, wet weight) was collected by SCUBA from Phuket Island at 1-5 m dept in May 2012. The tunicate sample was homogenized with 20 L of phosphate buffer solution (pH 7). Potassium cyanide solution (10%) was added to the homogenized solution to get 10 mM KCN at the final concentration, and the mixture was stirred for 5 hours. Then the mixture was macerated with methanol (5 x 20 L). The filtered solution was concentrated to be the aqueous emulsion, which was partitioned with EtOAc to give a dark-brown residue of the EtOAc extract (38.87 g). The extract was subjected to quick column chromatography (Silica gel, hexane-EtOAc 3:1 to 1:3, 100% EtOAc and methanol, respectively) and gel filtration chromatography (Sephadex LH 20, 100% methanol). The crude **1** was separated by a silica gel flash column (CH_2Cl_2-EtOAc 2:3). The precipitate of **1** was crystallized in co-solvent of CH_2Cl_2-methanol to give colorless crystals of Et 770 (258.2 mg, 3.97 x 10^{-3} % of wet wt).

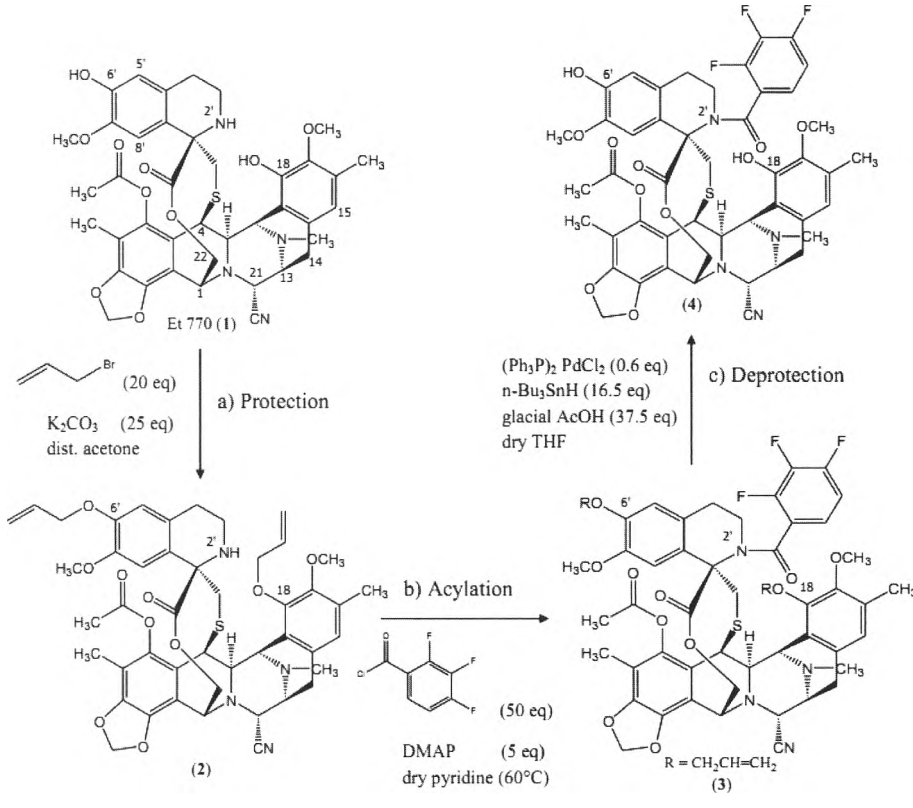
Preparation of 2'-N-(2,3,4-trifluorobenzoyl)Et 770 (Scheme 2) *a) 18,6'-O-bisallyl protection:* Et 770 (232.6 mg, 0.30 mmole) was dissolved in acetone (60 mL), then the solution was added K_2CO_3 (1.04 g, 25 eq) and stirred for 5 minutes at 0°C. Allyl bromide (522.7 µl, 20 eq) was added drop wise over 20 minutes to the vigorously stirred mixture. After the reaction suspension was stirred for 1 hour at 0°C, the reaction flask was further stirred at room temperature for 18 hours. Then, the reaction mixture was filtered and concentrated *in vacuo*, the residue was diluted with water (50 mL) and extracted with $CHCl_3$ (5 x 50 mL). The combined organic layer was washed with brine (50 mL), dried with anh. Na_2SO_4 and concentrated *in vacuo*. The crude extract was separated by a silica gel flash column using elution of gradient benzene-EtOAc 15:1 to 1:1 and 0:1 to provide 18,6'-*O*-bisallyl Et 770 (**2**) in 77.5% yield.

b) 2'-N acylation: Compound **2** (15.3 mg, 0.018 mmole) and DMAP (11 mg, 5 eq) were dissolved in pyridine (1.5 mL), and then the solution was stirred 5 minutes at 0°C. Then, 2,3,4-trifluorobenzoyl chloride (114 µL, 50 eq) was slowly dropped into the stirring cold solution. After the reaction solution was stirred for 30 minutes at 0°C, the reaction was further stirred at room temperature until the TLC showed disappearance of the starting material. After the solvent was removed *in vacuo*, the residue was diluted with water (10 mL) and extracted with CH_2Cl_2 (5 x 15 mL). The combined organic layer was washed with brine (40 mL) and dried *in vacuo* to give a residue. This residue was purified by a silica gel flash column using elution of gradient CH_2Cl_2-EtOAc 15:1 to 1:1 and 0:1 to provide 2'-*N*-(2,3,4-trifluorobenzoyl)-18,6'-*O*-bisallyl Et 770 (**3**) in 80.1% yield.

c) 18,6'-O-bisallyl deprotection: Tributyltin hydride (52 µL, 16.5 eq) was added dropwise over 10 minutes to a vigorously stirred solution of **3** (11.8 mg, 0.012 mmole), $(Ph_3P)_2PdCl_2$ (5 mg, 0.6 eq), and glacial AcOH (26 µL, 37.5 eq) in dry THF (4 mL) at 25°C, and the mixture was stirred at 25°C for 4 hours. Then, the mixture was diluted with water (10 mL), made alkaline with 5% aqueous $NaHCO_3$, and extracted with $CHCl_3$ (3 x 30 mL). The combined extract was washed with 5% aq. $NaHCO_3$ and dried *in vacuo* to give a residue. This residue was purified by a silica gel flash column using elution of gradient CH_2Cl_2-EtOAc 15:1 to 1:1 and 0:1 to provide 2'-*N*-(2,3,4-trifluorobenzoyl)Et 770 (**4**) in 31.5 % yield.

Spectroscopic data and physical properties of Et 770 and its derivatives Ecteinascidin 770 (**1**): Spectroscopic data and physical properties see *Ref.* 6) 18,6'-*O*-bisallyl Et 770 (**2**): Spectroscopic data and physical properties see *Ref.* 8) 2'-*N*-(2,3,4-trifluorobenzoyl)-18,6'-*O*-bisallyl Et 770 (**3**): Colorless amorphous powder, $[\alpha]_D^{25}$ -56.9° (c 0.58); IR (KBr) 3437, 2925, 2225, 1760, 1510, 1194 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ (ppm) 7.07, 7.05, 6.52, 6.33, 6.05, 6.05, 5.96, 5.96, 5.41, 5.29, 5.20, 4.76, 4.67, 4.64, 4.46, 4.35, 4.27, 4.14, 3.72, 3.59, 3.56, 3.50, 3.50, 3.45, 2.98, 2.89, 2.49, 2.49, 2.37, 2.26, 2.12, 2.01, 1.79; ^{13}C NMR ($CDCl_3$, 75 MHz) δ (ppm) 168.8, 168.3, 164.0, 150.2, 148.8, 147.5, 147.2, 145.5, 141.4, 140.9, 134.7, 133.0, 131.2, 130.0, 127.7, 126.0, 124.6, 123.3, 123.2, 122.4, 118.1, 117.9, 116.8, 113.4, 112.8, 112.6, 112.6, 111.0, 102.1, 72.9, 70.5, 69.7, 60.9, 60.6, 60.4, 59.4, 59.4, 55.5, 55.2, 54.9, 45.5, 42.2, 41.8, 39.1, 29.3, 24.9, 20.3, 15.6, 9.8; HR-FABMS m/z 1008.3228 (MH^+, calcd for $C_{53}H_{51}F_3N_4O_{10}S$, 1008.3227); CD Δε nm (c 10.9 µM, methanol, 22°C) 2.9 (210), -2.4 (216), -0.6 (226), 0.8 (256). 2'-*N*-(2,3,4-trifluorobenzoyl)Et 770 (**4**): Colorless amorphous powder, $[\alpha]_D^{25}$ -45.6° (c 0.09);

IR (KBr) 3436, 2925, 2854, 1759, 1629, 1462, 1196 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ (ppm) 7.07, 7.05, 6.42, 6.38, 6.07, 5.97, 5.76, 5.48, 4.69, 4.62, 4.39, 4.23, 3.66, 3.65, 3.58, 3.53, 3.42, 2.98, 2.57, 2.48, 2.31, 2.21, 2.03, 1.81; HR-FABMS m/z 928.2611 (MH^+, calcd for $C_{47}H_{43}F_3N_4O_{10}S$, 928.2601); CD Δε nm (c 19.4 μM, methanol, 22°C) 1.8 (201), -4.2 (216), -2.3 (226), 0.7 (251).



Scheme 2 General procedure for preparation of 2′-*N*-(2,3,4-trifluorobenzoyl)Et 770

RESULTS AND DISCUSSION

Preparation of 2′-*N*-(2,3,4-trifluorobenzoyl)Et 770 was achieved by a three-step transformation of Et 770, including: a) 18,6′-*O*-bisallyl protection, b) 2′-*N*-acylation, and c) 18,6′-*O*-bisallyl deprotection (**Scheme 2**). The reactive 18- and 6′-OHs of **1** were protected by using allyl bromide and K_2CO_3 to provide **2** in 77.5% yield. The ^1H- and ^{13}C-NMR spectra of **2** showed the characteristic signals similar to those of **1**. The appearance of the allyl-proton signals (around δ_H 5-6) along with the disappearance of the 18-OH (δ_H 5.77) and 6′-OH (δ_H 5.59) signals and the shifts of aromatic carbon signals implied the nucleophilic substitutions at the phenolic hydroxyls by the allyl groups. The 2′-*N* acylation of **2** was subsequently performed with 2,3,4-trifluorobenzoyl chloride and a catalyst DMAP to provide **3** in 80.1% yield. The ^1H- and ^{13}C-NMR spectra of **3** showed characteristic signals similar to those of **2**. The additional signals of the corresponding two aromatic protons at δ_H 7.07 and 7.05 and the additional carbonyl carbon at δ_C 168.8 implied the 2′-*N* substituted by a 2,3,4-trifluorobenzoyl group. For the deprotection step, the allyl groups were converted to the OH groups by using n-Bu_3SnH to donate hydride and using $(Ph_3P)_2PdCl_2$ as a catalyst to provide **4** in 31.5% yield. The ^1H- and ^{13}C-NMR spectra data of **4** showed characteristic signals similar to those of **3**. The disappearance of the allyl signals along with the appearance of the 18-OH (δ_H 5.76) and 6′-OH (δ_H 5.48) signals implied that the allyl groups were completely removed and replaced by the phenolic hydroxyl protons.

CONCLUSION

Et 770 (**1**) from the Thai tunicate *Ecteinascidia thurstoni* is a potent cytotoxic compound. To improve its biological activity, the chemical modification of **1** has been focused. We were able to prepare additional 2′-*N*-2,3,4-trifluorobenzoyl derivative of **1** via the three-step transformation. In the next step, its cytotoxicity by measuring IC_{50} values against human colon carcinoma (HCT116), lung carcinoma (QG56) and prostate carcinoma (DU145) cell lines will be evaluated.

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