

Tryprostatis A and B, Novel Mammalian Cell Cycle Inhibitors Produced by *Aspergillus fumigatus*

Sir:

In the course of our screening for new inhibitors of mammalian cell cycle¹⁾, we have discovered two novel compounds named tryprostatis A (**1**) and B (**2**) (Fig. 1), from the secondary metabolites of a marine fungal strain BM939. The producing strain was isolated from a sea sediment sample collected in the sea bottom (760 meters deep) of the mouth of Oi river, Sizuoka prefecture, Japan, and was identified as *Aspergillus fumigatus* through a taxonomic study. Here we preliminarily report the isolation, structures and biological activities of **1** and **2**.

The producing strain was cultured in a 30-liter jar fermenter containing 18 liters of fermentation medium (glucose 3%, soluble starch 2%, soybean meal 2%, K₂HPO₄ 0.5% and MgSO₄·7H₂O 0.05%, adjusted at pH 6.5 before sterilization) containing 0.05% of CA-123 and KM-68 antifoam, respectively. The fermentation was carried out for 72 hours at 28°C under the following condition; 350 rpm stirring speed and 7 liters/minute aeration rate.

The fermentation and the following separation procedure were monitored by the inhibitory activity on the cell cycle progression of a mouse tsFT210 cell line which is a temperature-sensitive p34^{cdc2} mutant. The cells were growing normally at 32°C, but arrested in the G2 phase at 39°C¹⁻³⁾.

The cultured whole broth was centrifuged to separate

Fig. 1. Structures of tryprostatis A (**1**) and B (**2**).

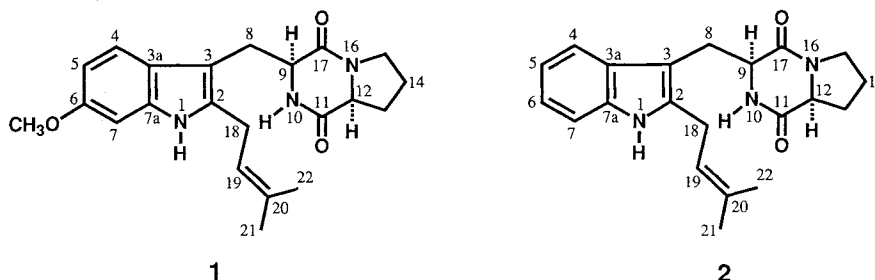


Fig. 2. Purification procedure for tryprostatis A (**1**) and B (**2**).

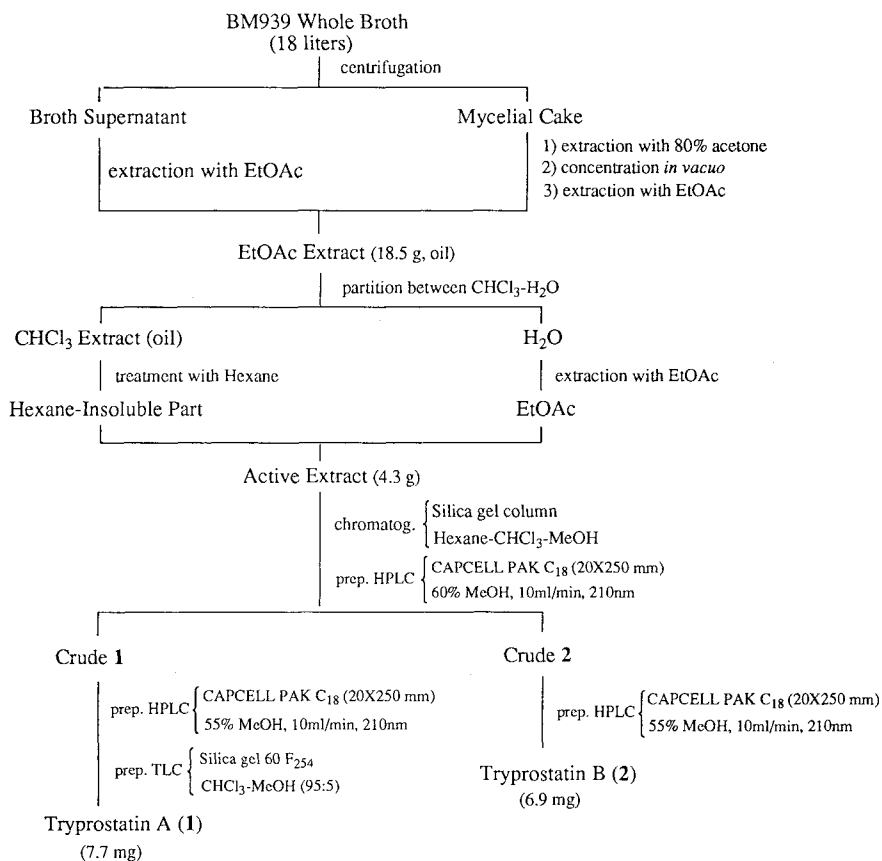


Table 1. Physico-chemical properties of tryprostatis A (1) and B (2).

Characteristics	1	2
Appearance	Pale yellow crystalline solid	Pale yellow crystalline solid
MP	120-123°C	102-105°C
$[\alpha]_D^{27}$	-69.7°(c 0.70, CHCl ₃)	-71.1°(c 0.63, CHCl ₃)
Molecular Formula	C ₂₂ H ₂₇ N ₃ O ₃	C ₂₁ H ₂₅ N ₃ O ₂
HR-EI-MS	M ⁺	M ⁺
Found (<i>m/z</i>)	381.2050	351.1943
Calcd (<i>m/z</i>)	381.2050	351.1944
UV $\lambda_{\max}^{\text{MeOH}}$ nm (ϵ)	227 (24540) 270 (5450) 297 (6590)	226 (23780) 277 (8690) 298 (sh, 7180)
IR ν_{\max}^{KBr} cm ⁻¹	3365, 3270, 3070, 2965, 2930, 2880, 2860, 1670, 1655, 1460, 1430, 1160	3370, 3270, 3070, 2970, 2930, 2875, 2860, 1670, 1655, 1460, 1435, 1160

Fig. 3. UV spectra of tryprostatis A (1) and B (2).

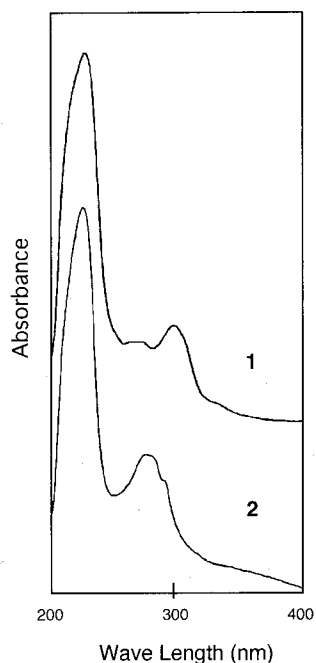
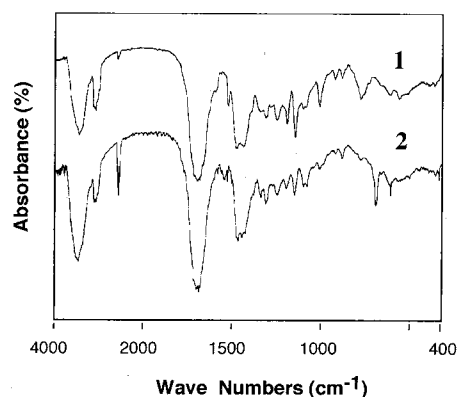


Fig. 4. IR spectra of tryprostatis A (1) and B (2).

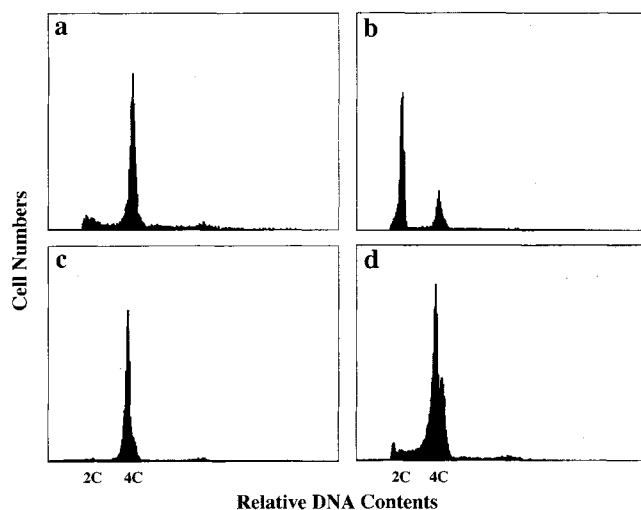


to a broth supernatant (18 liters) and a mycelial cake. The latter was extracted with 80% aqueous acetone which was evaporated *in vacuo* to remove acetone. Both the broth supernatant and the mycelium extract showed an inhibitory activity on the cell cycle progression of tsFT210 cells and thus both were extracted respectively with the same volume of EtOAc. The EtOAc solution obtained was combined and concentrated *in vacuo* to afford an oily extract (18.5 g) which was further purified as shown in Fig. 2 to give an active extract (4.3 g). This extract was then separated by a combination of column

chromatography (silica gel 60, Merck), repeated HPLC (CAPCELL PAK C₁₈, Shiseido) and preparative TLC (silica gel 60 F₂₅₄ plate, Merck) to obtain 1 (7.7 mg) and 2 (6.9 mg) (Fig. 2).

Tryprostatis A (1) and B (2) were obtained both as pale yellowed crystalline solids. The physico-chemical properties of 1 and 2 are summarized in Table 1. Their molecular formulae, C₂₂H₂₇N₃O₃ for 1 and C₂₁H₂₅N₃O₂ for 2, were determined by HR-EI-MS measurements (Found *m/z* 381.2050 (M⁺), Calcd for C₂₂H₂₇N₃O₃ 381.2050 for 1; Found *m/z* 351.1943 (M⁺), Calcd for C₂₁H₂₅N₃O₂ 351.1944 for 2). The UV spectra (Fig. 3) of 1 and 2 respectively revealed the presence of a 6-*O*-methylindole chromophore in 1 with the absorption maxima at 227 (ϵ 24540), 270 (5450) and 297 nm (6590)⁴ and the presence of an indole chromophore in 2 with 226 (23780), 277 (8690) and 298 (sh, 7180)⁵. And their IR spectra closely resembled in the functional group region, as shown in Fig. 4. The multiple absorption at

Fig. 5. Effects of **1** and **2** on cell cycle progression of tsFT210 cells.



The tsFT210 cells were synchronized in the G2 phase by incubation at 39.4°C for 17 hours at a density of 2×10^5 cells/ml in RPMI-1640 medium supplemented with 5% calf serum. Then, the cells were maintained at 39.4°C up to 4 hours (a) or allowed to progress into mitosis at 32°C for 4 hours in the absence (b) or in the presence of tryprostatin A (50 $\mu\text{g}/\text{ml}$) (c) and tryprostatin B (12.5 $\mu\text{g}/\text{ml}$) (d), respectively.

1670 and 1655 cm^{-1} due to amide groups together with the absence of the amide II band near 1550 cm^{-1} suggest the presence of diketopiperazine system^{5~7)} in both **1** and **2**. Eventually, the structures of tryprostatins A and B were determined as shown in Fig. 1 by detailed analyses of their ^1H and ^{13}C NMR spectra with the aid of 2D NMR techniques including field-gradient hetero-nuclear multiple-bond correlation (FG-HMBC) spectroscopy.

The molecules of **1** and **2** are composed from a 2-isoprenyltryptophan moiety and a proline residue, forming a diketopiperazine unit, which are distinguished from the molecules of fumitremorgin series⁸⁾ in the respect of opening the centered heterocyclic ring at C–N bond between 18 and 10 positions. Only few kinds of natural products structurally related to **1** and **2** such as deoxybrevianamide-E had so far been reported⁵⁾, and present result provides the first example of natural product belonging to this novel class as the inhibitor of mammalian cell cycle. Tryprostatins A and B may be shunt metabolites of verruculogen⁸⁾ and fumitremorgin series⁸⁾ like as deoxybrevianamide-E in biogenesis of brevianamides⁷⁾.

Tryprostatins A (**1**) and B (**2**) completely inhibited cell cycle progression of tsFT210 cells in the G2/M phase at final concentrations of 50 $\mu\text{g}/\text{ml}$ of **1** and 12.5 $\mu\text{g}/\text{ml}$ of **2**, respectively (Fig. 5). Further studies on their biological activities and mechanism of their action are currently undertaken.

Details of the structural and biological studies will be reported elsewhere.

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