Antitumor Effect of a Novel Phenanthroindolizidine Alkaloid Derivative Through Inhibition of Protein Synthesis

SATOSHI UENO, RYUTA YAMAZAKI, TAKASHI IKEDA, TAKASHI YAEGASHI and TAKESHI MATSUZAKI

Yakult Central Institute for Microbiological Research, Yakult Honsha Co., Ltd., Kunitachi, Tokyo, Japan

Abstract. *Aim: The present study aimed to determine the antitumor efficacy of a new Phenanthroindolizidine alkaloid (PA) derivative, YPC-10157, and to elucidate its mechanism of action. Materials and Methods: The in vitro and in vivo antitumor activity of YPC-10157 was evaluated against several human cancer cell lines and mouse xenograft models, respectively. Cell apoptosis was determined by measuring caspase-3/7 activity. The effect on protein synthesis was assessed using an in vitro cell-free translation assay system. Results: In vitro, YPC-10157 exhibited marked cell growth inhibition and induced apoptosis. In vivo, YPC-10157 had a strong antitumor effect on xenograft models of human colon cancer and leukemia. Moreover, YPC-10157 and its derivatives inhibited protein synthesis and their inhibitory activity on protein synthesis significantly correlated regarding cell growth. Conclusion: YPC-10157 has promising antitumor effects and suggest that its cytotoxic mechanism might involve the inhibition of protein synthesis.*

The class of compounds known as phenanthroindolizidine alkaloids (PAs), many of which have been isolated from plants of the Asclepiadaceae family, have been investigated extensively for their anti-inflammatory (1-3) and antitumor (4-24) activity. These compounds were expected to be candidates for anticancer drugs. Tylophorine and its analogs were assessed using a panel of 60 tumor cell lines at the National Cancer Institute and showed strong growth-inhibitory activity toward almost all the cell lines, including P-glycoprotein-overexpressing cells. Moreover, since the spectra of their inhibitory activity were unique among current antitumor compounds, these PAs were expected to become a new class of anticancer agents (4-10). In the 1960s, however, a tylophorine-related compound, tylocrebrine, failed in clinical trials because of side-effects on the central nervous system. Since then, there have been many attempts to improve the physical properties of PAs and to prevent them from passing through the blood–brain barrier (4).Various derivatives of PA derived from natural products have been synthesized and evaluated for antitumor properties *in vitro* and *in vivo*. While many of these PA analogs exhibited potent cytotoxic effects against human cancer cell lines *in vitro*, most of them were inefficacious *in vivo*, mainly due to poor pharmacokinetic profiles, general toxicity, low water solubility and so on. It has taken a lot of effort to improve their drug-like characteristics to develop them as anticancer drugs. Regarding the mechanism of growth inhibition, it was reported that these compounds inhibited the transcriptional activities of activator protein-1 (AP1) (4), nuclear factor-kappa B (NF-κB) (4-6) and hypoxia inducible factor-1 (HIF1) (7), key transcription factors in cancer cell proliferation, differentiation, resistance to chemotherapy *etc*. It was also described that they suppressed protein kinase B, also known as AKT, signal activation (5) and induce cell-cycle arrest by modulating cell-cycle regulatory proteins (8-10). Furthermore, it was reported that they interfered with DNA biosynthesis by inhibiting the activity of thymidylate synthase/dihydrofolate reductase (11-13), and could also block protein biosynthesis by affecting the peptide chain elongation step (14-16).We synthesized several derivatives (17-19) of a PA isolated from *Ideopsis similis*, a butterfly found in Okinawa, Japan (20). Some of these compounds exhibited very strong growth-inhibitory activity against human cancer cell lines *in vitro* and also showed effective antitumor activity against xenograft tumors in nude mice (17-19). However, their detailed antitumor effects and mechanism of action were unclear. In the present report, we focus on a novel PA derivative, YPC-10157 (Figure 1), and evaluate its cytotoxic effect on several human cancer cell lines and its antitumor effect against human cancer xenograft models. Additionally, to elucidate its mechanism of action, we further investigated its inhibitory effect on protein synthesis as a possible mechanism.

Correspondence to: Satoshi Ueno, Yakult Central Institute for Microbiological Research, Yakult Honsha Co., Ltd., 1796 Yaho, Kunitachi, Tokyo, 186-8650, Japan. Tel: +81 425778970, Fax: +81 425773020, e-mail: satoshi-ueno@yakult.co.jp

Key Words: Phenanthroindolizine alcaloid, caspase activity, YPC-10157, antitumor activity.

Table I. *Growth inhibition by 96 h treatment of YPC-10157 in human cancer cell lines.*

	Cell line							
	A549	HT-29	HCT116	MCF7	HCC1806	MDA-MB-231	MDA-MB-468	HL-60
IC_{50} (nM)	0.728	3.00	0.687	2.84	1.99	0.609	2.08	0.419

IC_{50}: Half maximal inhibitory concentration.

Materials and Methods

Reagents. YPC-10157 and other PA derivatives (Figure 1) were synthesized in our laboratory (18-20). Cycloheximide was purchased from Calbiochem (La Jolla, CA, USA). For *in vitro* assays, all compounds were dissolved in dimethylsulfoxide (DMSO) as a stock solution and the final concentration of DMSO was less than 0.1%.

Cell culture. All human cancer cell lines were purchased from the American Type Culture Collection (Manassas, VA, USA). Human lung cancer cell line A549, colon cancer HT-29 and HCT116 cells were maintained in Dulbecco's modified Eagle's medium (Invitrogen, Carlsbad, CA, USA). Breast cancer MCF7, HCC1806, MDA-MB-231 and MDA-MB-468, promyelocytic leukemia HL-60 were maintained in RPMI medium 1640 (Invitrogen). Each medium was supplemented with 10% heat-inactivated fetal bovine serum (Thermo Fisher Scientific, Waltham, MA, USA) , 100 units/ml penicillin and 100 μg/ml streptomycin (Invitrogen), and cells were grown at 37˚C in a humidified atmosphere of 5% CO_2 and 95% air.

Cell viability assay. Cell viability was assayed in a 96-well plate using a TetraColor ONE (Seikagaku Corp., Tokyo, Japan), according to the manufacturer's protocol. Briefly, exponentially growing cells were seeded at a density of 1×10^3 cells/well. The next day, serially diluted compounds were added to each well. After 96 h of incubation, 2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium monosodium salt, WST-8, was added to each well and the plates were incubated at 37˚C for 1 h. Absorbance was then measured at 450 nm using a SPECTRA Max PLUS384 (Molecular Devices, Sunnyvale, CA, USA). The half maximal (50%) inhibitory concentration (IC_{50}) for cell viability was defined as the concentration of compound that inhibited cell viability by 50% compared to solvent-treated control cells.

Caspase-3/7 activity assay. Caspase-3/7 activities in the cells were measured with Caspase-Glo 3/7 Assay Kit (Promega, Madison, WI, USA) according to the manufacturer's instructions. Cells were seeded in a 96-well plate at 5×10^3 cells/well, and then treated with YPC-10157 for 24 h. Treated cells were incubated with 10 μl of the assay reagent at room temperature. After 1 h, luminescence was measured to calculate caspase-3/7 activities.

In vivo tumor xenograft model. Six-week-old male BALB/c nude mice were purchased from Japan SLC, Inc. (Shizuoka, Japan). Cells were suspended in saline (2×10^7 cells/ml) and 0.1 ml of the cell suspension was injected subcutaneously into the right flank of each mouse. For the HCT-116 xenograft model, when tumors reached a mean volume of 160 to 260 mm^3 (day 1), mice were randomly divided into three groups of five mice per group; YPC-10157 mesylate was dissolved in 5% glucose and administered intravenously at 22.2 mg/kg or 44.4 mg/kg on days 1, 3, 5, 8, 10, 12, 15, and 17. For the HL-60 xenograft model, when tumors reached a mean volume of 90 to 240 mm^3 (day 1), mice were divided into three groups of four mice per group; YPC-10157 mesylate was administered intravenously at 16.7 mg/kg or 33.3 mg/kg on days 2, 6, and 10.

Tumor size was measured using calipers twice a week and the body weight of each mouse was monitored for assessing toxicity. Tumor volume was estimated by using the formula length×$width^2\times1/2$. On day 22 for the HCT116 model, and day 16 for the HL60 model, the antitumor activity was evaluated by weighing the tumor tissues. The tumor growth inhibition rate, IR (%), was calculated as [1−(average tumor weight of each treated group)/(average tumor weight of control group)]×100.

All experiments with animals were conducted in accordance with the Guidelines of the Yakult Central Institute for Microbiological Research and protocols approved by The Institutional Animal Care and Use Committee of the Yakult Central Institute (Approved No. 10-0224).

Cell-free translation assay. A cell-free translation assay was carried out using the TNT^R Coupled Reticulocyte Lysate System (Promega), according to the manufacturer's directions. Briefly, firefly luciferase cDNA as a template and compounds were added directly to the TNT^R Lysate System and incubated for 1.5 h at 30˚C. Firefly luciferase activity was measured using the Steady-GloR Luciferase assay system (Promega) with SPECTRA Max M5e (Molecular Devices). The IC_{50} for protein synthesis was defined as the concentration of compound that inhibited protein synthesis by 50% compared to the solvent-treated control.

Statistical analysis. For comparisons of more than two groups, the data were analyzed for statistical significance with Dunnett's multiple comparison test. For the correlation analysis, the correlation coefficient was calculated by the linear least-squares regression method. Probability values of less than 0.05 were considered significant.

Results

Inhibitory effect of YPC-10157 on growth of human cancer cell lines in vitro. The effect of YPC-10157 on cell proliferation after 96 h treatment was examined in eight human cancer cell lines: A549, HT-29, HCT116, MCF7, HCC1806, MDA-MB-231, MDA-MB-468 and HL-60. As shown in Table I, YPC-10157 inhibited the growth of all cell

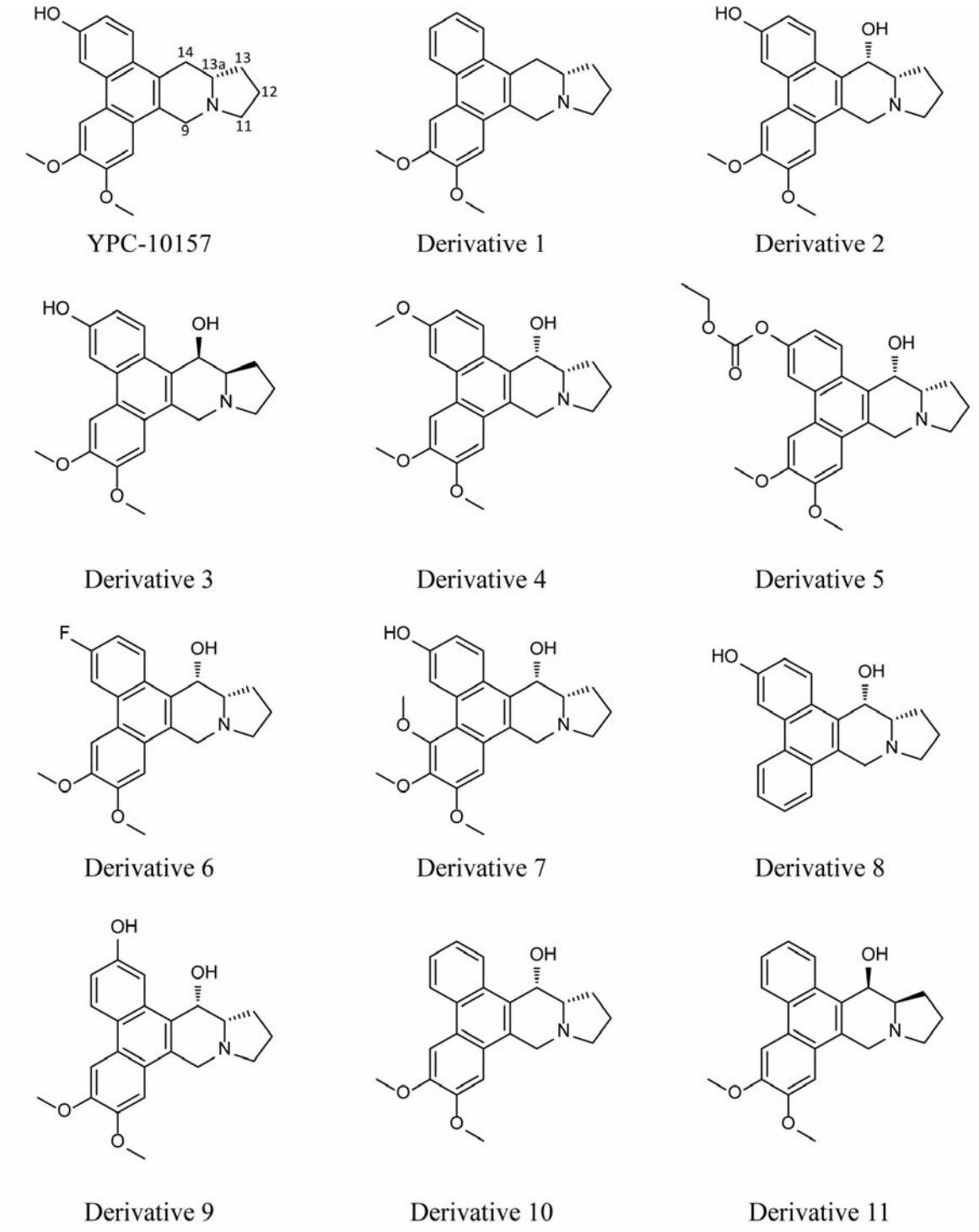

Figure 1. *Chemical structure of YPC-10157 and its derivatives*.

lines in the lower nanomolar range. Out of all the cell lines used in this study, HL-60 showed the highest sensitivity to YPC-10157, with an IC_{50} of 0.419 nM.

Activation of apoptotic pathway by YPC-10157. Caspases are the main enzymes that mediate apoptosis. To determine whether YPC-10157 inhibits the growth of cancer cells *via*

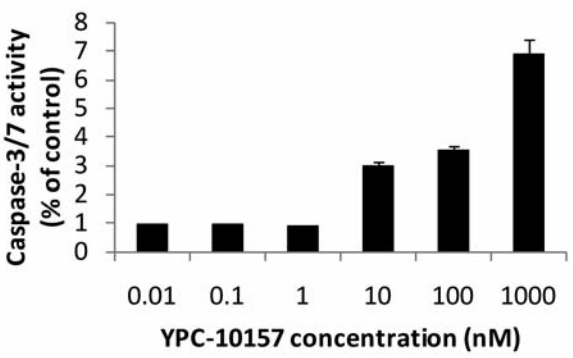

Figure 2. *Effect of YPC-10157 on activation of caspase-3 and -7. HL-60 cells were treated with YPC-10157 for 24 h. Activities of caspase-3 and -7 were measured using Caspase-Glo 3/7 assay. Data are expressed as mean±S.D. of triplicate samples.*

Table II. *IC_{50} values of YPC-10157 and its derivatives for cell growth and protein synthesis.*

Sample	IC_{50} (nM)		
	Cell growth[1]		Protein synthesis[2]
	A549	HT-29	
YPC-10157	0.6	1.6	107.4
Derivative 1	1.9	8.5	253.4
Derivative 2	0.5	1.2	36.9
Derivative 3	8.1	40.8	479.9
Derivative 4	0.3	1.9	30.5
Derivative 5	0.1	0.1	61.3
Derivative 6	10.3	17.8	463.1
Derivative 7	1298.9	1474.8	6399.0
Derivative 8	315.9	409.6	4140.0
Derivative 9	4.3	6.2	566.0
Derivative 10	6.8	9.5	146.8
Derivative 11	546.8	531.2	2055.9

[1]Cells were treated with compounds for 96 h. [2]The reaction mixture and compounds were incubated for 1.5 h at 30˚C.

the apoptotic pathway, we measured changes in the caspase activity in HL-60 cells after 96 h treatment. As shown in Figure 2, YPC-10157 treatment dose-dependently increased caspase-3 and -7 activities.

Antitumor effect of YPC-10157 on tumor xenografts in vivo. We further evaluated the antitumor activity of YPC-10157 in human cancer cell xenografts using HCT116 and HL-60 which showed higher sensitivity to YPC-10157 *in vitro* (Table I). In the HCT116 model, treatment with YPC-10157 mesylate at 22.2 or 44.4 mg/kg significantly inhibited the growth of tumors, with IR values of 49.4% and 64.6%, respectively, compared to the vehicle-only on day 22 (Figure 3A). Although the administration of YPC-10157 at 44.4 mg/kg led to 15% body weight loss compared with day 1, the body weight recovered after the treatment ceased. In the HL-60 model, treatment with YPC-10157 at 16.7 or 33.3 mg/kg more markedly inhibited the growth of tumors, with IR values of 57.4% and 96.8%, respectively, compared to the vehicle on day 16 (Figure 3B). In particular, YPC-10157 at 33.3 mg/kg reduced tumor volume on day 13 compared to day 1. No body weight loss was observed during the treatment of animals of this model.

Effect of YPC-10157 on protein synthesis. Next, we investigated the mechanism of the growth-inhibitory effect. As previously mentioned, it seemed tylophorine and its analogs have several mechanisms of action (4-16). Because it was also reported that similar structural analogs had different potency or selectivity under each mechanism of action (21), how YPC-10157 inhibited growth was obscure. Therefore, we investigated its effect on protein synthesis as a possible mechanism of its cytotoxic action. To elucidate whether YPC-10157 directly affects protein synthesis, we performed an *in vitro* cell-free translation assay using rabbit reticulocyte lysate (22). YPC-10157 exhibited a dose-dependent inhibition of protein synthesis as effectively as cycloheximide, a protein synthesis inhibitor (Figure 4).

Correlation between the cell-growth inhibition and protein synthesis inhibition of PA derivatives. To further confirm the inhibitory activity of YPC-10157 on protein synthesis associated with its cytotoxic action, we examined the inhibitory activities of several YPC-10157-based PA derivatives (Figure 1) on the protein synthesis and cell growth of A549 and HT-29 cells. All the derivatives, including YPC-10157, inhibited both the growth of cancer cell lines and protein synthesis (Table II), but their inhibitory potencies differed. Therefore, to examine the correlation between the cell growth inhibition and protein synthesis inhibition, IC_{50} values for cell growth inhibition of all derivatives were plotted against those for protein synthesis inhibition (Figure 5). As a result, protein synthesis inhibition was significantly correlated with growth inhibition in both A549 cells (Figure 5A; r=0.95, $p<0.001$) and in HT-29 cells (Figure 5B; r=0.92, $p<0.001$).

Discussion

YPC-10157 is a novel PA derivative optimized for anti-proliferative activity and water solubility. YPC-10157 showed potent growth-inhibitory activity against all tested human cell lines derived from lung cancer, colon cancer,

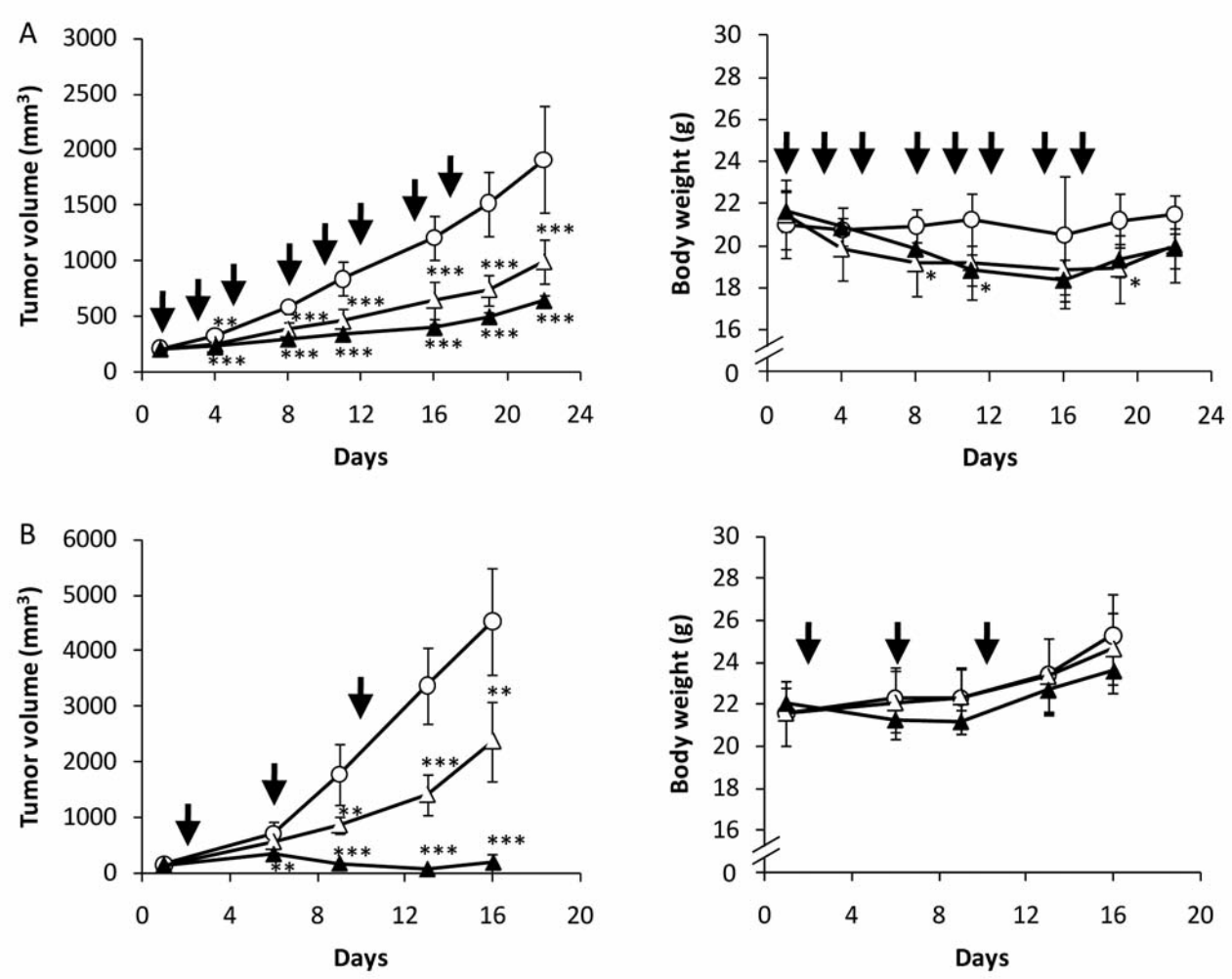

Figure 3. *Antitumor activity of YPC-10157 mesylate in vivo. A: BALB/c nude mice (n=5/group) were inoculated subcutaneously with HCT116 cells. After tumors had formed, the mice were treated intravenously with YPC-10157 mesylate at 22.2 (white triangles) or 44.4 (black triangles) mg/kg or with vehicle-only (white circles). Treatment was given on days 1, 3, 5, 8, 10, 12, 15, and 17 (arrows). B: Mice bearing HL-60 xenograft tumors (n=4/group) were treated intravenously either with YPC-10157 mesylate at 16.7 (open triangles) or 33.3 (closed triangles) mg/kg or with vehicle-only (open circles). Treatment was given on days 2, 6, and 10 (arrows). Data are expressed as mean±S.D. *p<0.05, **p<0.01, ***p<0.001 vs. vehicle control.*

breast cancer and leukemia, through an apoptotic mechanism, *in vitro* (Table I, Figure 2). These results suggest that YPC-10157 may have therapeutic utilities against a broad spectrum of cancer types.

Some reports have indicated that a hydroxyl moiety on C14 of the phenanthroindolizidine structure is important for strong antitumor activity *in vivo* (4, 23) and that the methoxy moiety on the phenanthrene ring may contribute to less cytotoxicity *in vitro* (24) and metabolic instability *in vivo* (23). In the case of YPC-10157, although it lacks a hydroxyl moiety at C14 and it has two methoxy moieties on its phenanthrene ring, it exhibited a strong antitumor effect with mild toxicity *in vivo* (Figure 3). Our previous study had also shown that YPC-10157 has more potent antitumor activity and lower lethal toxicity than its hydroxylated derivatives (17). These results indicate that YPC-10157 has more favorable drug-like properties than previously reported PA derivatives.

In the cell-free translation assay, YPC-10157 dose-dependently inhibited protein synthesis (Figure 4). In the correlative evaluation, YPC-10157 and its derivatives inhibited both cancer cell growth and protein synthesis (Table II), and these activities correlated significantly with each other (Figure 5). Previous reports suggested that PA and its derivatives have various mechanisms of action (4-16). A recent report described that phenanthrene-based analogs, being structurally fairly

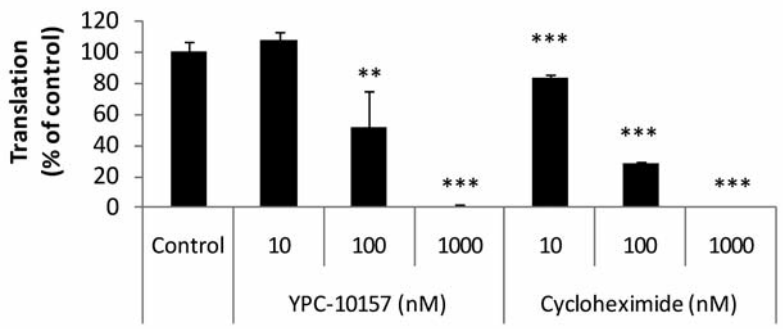

Figure 4. *Dose-responsive inhibitory effect of YPC-10157 on protein synthesis. Firefly luciferase cDNA as a template with YPC-10157 or cycloheximide was added directly to the reaction system and incubated for 1.5 h at 30˚C. Protein synthesis efficiency was measured as firefly luciferase activity as described in the Materials and Methods. Data are expressed as the mean±S.D. of triplicate samples. **p<0.01, ***p<0.001 vs. dimethylsulfoxide treated control.*

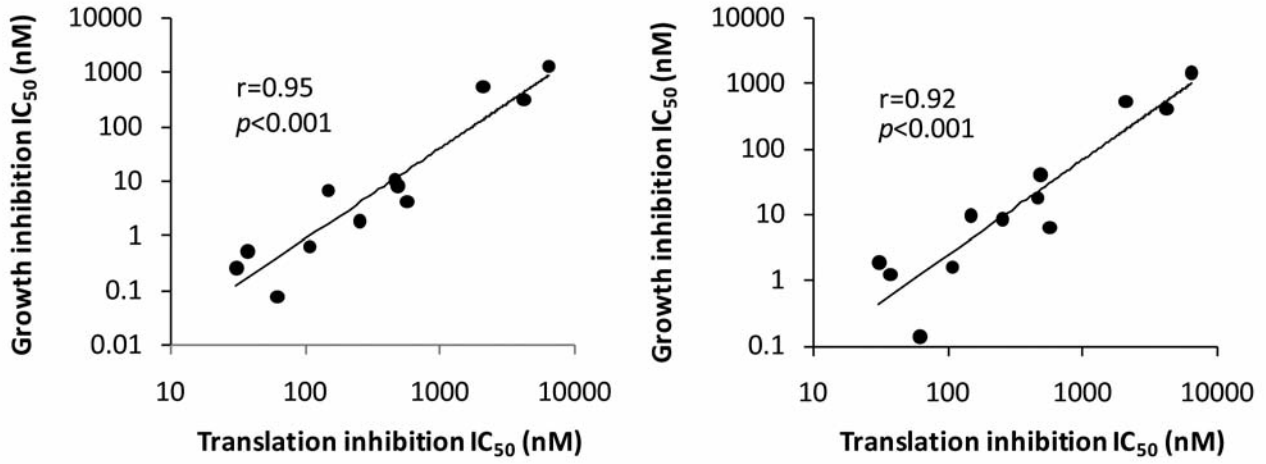

Figure 5. *Correlation between the cell growth inhibition and protein synthesis inhibition by YPC-10157 and its derivatives. The half maximal inhibitory concentration (IC_{50}) for protein synthesis and that for cell growth in cultures of A549 (A) and HT-29 (B) cells was plotted on a double logarithmic graph. The correlation coefficient was calculated by linear least-squares regression.*

similar, exhibit different mechanisms of action (21). Our study suggests that these PA derivatives, including YPC-10157, exhibit antitumor activity by inhibiting protein synthesis. It has been reported that tylophorines inhibit protein synthesis by blocking chain elongation (14). It seems likely YPC-10157 and its derivatives have a similar mechanism of action, but further study is needed. In conclusion, a novel PA derivative, YPC-10157, has strong anticancer activity *in vitro* and *in vivo*, and its mechanism of action might be through disruption of protein synthesis. These results indicate that YPC-10157 may be a potential candidate for anticancer therapy.

Acknowledgements

We appreciate the technical assistance of Ms. Yukiko Nishiyama.

References

1 Gopalakrishnan C, Shankaranarayanan D, Nazimudeen SK and Kameswaran L: Effect of tylophorine, a major alkaloid of *Tylophora indica*, on immunopathological and inflammatory reactions. Indian J Med Res *71*: 940-948, 1980.

2 Yang CW, Chen WL, Wu PL, Tseng HY and Lee SJ: Anti-inflammatory mechanisms of phenanthroindolizidine alkaloids. Mol Pharmacol *69*: 749-758, 2006.

3 Yang CW, Chuang TH, Wu PL, Huang W H and Lee SJ: Anti-inflammatory effects of 7-methoxycryptopleurine and structure–activity relations of phenanthroindolizidines and phenanthroquinolizidines. Biochem Biophys Res Commun *354*: 942-948, 2007.

4 Gao W, Lam W, Zhong S, Kaczmarek C, Baker DC and Cheng YC: Novel mode of action of tylophorine analogs as antitumor compounds. Cancer Res *64*: 678-688, 2004.

5 Lin JC, Yang SC, Hong TM, Yu SL, Shi Q, Wei L, Chen HY, Yang PC and Lee KH: Phenanthrene-based tylophorine-1 (PBT-1) inhibits lung cancer cell growth through the Akt and NF-κB pathways. J Med Chem *52*: 1903-1911, 2009.
6 Shiah HS, Gao W, Baker DC and Cheng YC: Inhibition of cell growth and nuclear factor-κB activity in pancreatic cancer cell lines by a tylophorine analogue. DCB-3503, Mol Cancer Ther *5*: 2484-2493, 2006.
7 Cai XF, Jin X, Lee D, Yang YT, Lee K, Hong YS, Lee JH and Lee JJ: Phenanthro- quinolizidine alkaloids from the roots of *Boehmeria pannosa* potently inhibit hypoxia-inducible factor-1 in AGS human gastric cancer cells. J Nat Prod *69*: 1095-1097, 2006.
8 Wu CM, Yang CW, Lee YZ, Chuang TH, Wu PL, Chao YS and Lee SJ: Tylophorine arrests carcinoma cells at G1 phase by down-regulating cyclin A2 expression. Biochem Biophys Res Commun *386*: 140-145, 2009.
9 Lee SK, Nam KA and Heo YH: Cytotoxic activity and G2/M cell cycle arrest mediated by antofine, a phenanthroindolizidine alkaloid isolated from *Cynanchum paniculatum*. Planta Med *69*: 21-25, 2003.
10 Min HY, Chung HJ, Kim EH, Kim S, Park EJ and Lee SK: Inhibition of cell growth and potentiation of tumor necrosis factor-α (TNF-α)-induced apoptosis by a phenanthroindolizidine alkaloid antofine in human colon cancer cells. Biochem Pharmacol *80*: 1356-1364, 2010.
11 Rao KN and Venkatachalam SR: Inhibition of dihydrofolate reductase and cell growth activity by the phenanthroindolizidine alkaloids pergularinine and tylophorinidine: the *in vitro* cytotoxicity of these plant alkaloids and their potential as antimicrobial and anticancer agents. Toxicol In Vitro *14*: 53-59, 2000.
12 Rao KN, Bhattacharya RK and Veankatachalam SR: Inhibition of thymidylate synthase by pergularinine, tylophorinidine and deoxytubulosine. Indian J Biochem Biophys *36*: 442-448, 1999.
13 Rao KN, Bhattacharya RK and Venkatachalam SR: Thymidylate synthase activity in leukocytes from patients with chronic myelocytic leukemia and acute lymphocytic leukemia and its inhibition by phenanthroindolizidine alkaloids pergularinine and tylophorinidine. Cancer Lett *128*: 183-188, 1998.
14 Huang MT and Grollman AP: Mode of action of tylocrebrine: effects on protein and nucleic acid synthesis. Mol Pharmacol *8*: 538-550, 1972.
15 Donaldson GR, Atkinson MR and Murray AW: Inhibition of protein synthesis in Ehrlich ascites tumour cells by the phenanthrene alkaloids tylophorine, tylocrebrine and cryptopleurine. Biochem Biophys Res Commun *31*: 104-109, 1968.
16 Gupta RS and Siminovitch L: Mutants of CHO cells resistant to the protein synthesis inhibitors, cryptopleurine and tylocrebrine: genetic and biochemical evidence for common site of action of emetine, cryptopleurine, tylocrebine, and tubulosine. Biochemistry *16*: 3209-3214, 1977.
17 Ikeda T, Yaegashi T, Matsuzaki T, Yamazaki R, Hashimoto S and Sawada S: Synthesis of phenanthroindolizidine alkaloids and evaluation of their antitumor activities and toxicities. Bioorg Med Chem Lett *19*: 5978-5981, 2011.
18 Ikeda T, Yaegashi T, Matsuzaki T, Hashimoto S and Sawada S: Asymmetric synthesis of phenanthroindolizidine alkaloids with hydroxyl group at the C14 position and evaluation of their antitumor activities. Bioorg Med Chem Lett *21*: 342-345, 2011.
19 Ikeda T, Yaegashi T, Matsuzaki T, Yamazaki R, Ueno S, Hashimoto S and Sawada S: Synthesis of phenanthroindolizidine alkaloids with an acyloxy group at the C3 position and their antitumor activities and toxicities. Lett Drug Des Discov *9*: 447-453, 2012.
20 Komatsu H, Watanabe M, Ohyama M, Enya T, Koyama K, Kanazawa T, Kawahara N, Sugimura T and Wakabayashi K: Phenanthroindolizidine alkaloids as cytotoxic substances in a Danaid butterfly, *Ideopsis similis*, against human cancer cells. J Med Chem *44*: 1833-1836, 2001.
21 Gao W, Chen AP, Leung CH, Gullen EA, Fürstner A, Shi Q, Wei L, Lee KH and Cheng YC: Structural analogs of tylophora alkaloids may not be functional analogs. Bioorg Med Chem Lett *18*: 704-709, 2008.
22 Kim JT, Kim KD, Song EY, Lee HG, Kim JW, Kim JW, Chae SK, Kim E, Lee MS, Yang Y and Lim JS: Apoptosis-inducing factor (AIF) inhibits protein synthesis by interacting with the eukaryotic translation initiation factor 3 subunit p44 (eIF3g). FEBS Lett *580*: 6375-6383, 2006.
23 Fu Y, Lee SK, Min HY, Lee T, Lee J, Cheng M and Kim S: Synthesis and structure-activity studies of antofine analogues as potential anticancer agents. Bioorg Med Chem Lett *17*: 97-100, 2007.
24 Gao W, Bussom S, Grill SP, Gullen EA, Hu YC, Huang X, Zhong S, Kaczmarek C, Gutierrez J, Francis S, Baker DC, Yu S and Cheng YC: Structure-activity studies of phenanthroindolizidine alkaloids as potential antitumor agents. Bioorg Med Chem Lett *17*: 4338-4342, 2007.

Received March 20, 2014
Revised May 21, 2014
Accepted May 22, 2014