PHYTOTHERAPY RESEARCH
Phytother. Res. **25**: 147–150 (2011)
Published online 9 July 2010 in Wiley Online Library
(wileyonlinelibrary.com) **DOI**: 10.1002/ptr.3219

SHORT COMMUNICATION

Cryptolepine, Isolated from *Sida acuta*, Sensitizes Human Gastric Adenocarcinoma Cells to TRAIL-induced Apoptosis

Firoj Ahmed,[1,2] Kazufumi Toume,[1] Takashi Ohtsuki,[1] Mahmudur Rahman,[2] Samir Kumar Sadhu[2] and Masami Ishibashi[1]*

[1]Graduate School of Pharmaceutical Sciences, Chiba University,1-33Yayoi-cho, Inage-ku, Chiba 263-8522, Japan
[2]Pharmacy Discipline, Life Science School, Khulna University, Khulna-9208, Bangladesh

Bioassay guided separation of *Sida acuta* whole plants led to the isolation of an alkaloid, cryptolepine (1), along with two kaempferol glycosides (2–3). Compound 1 showed strong activity in overcoming TRAIL-resistance in human gastric adenocarcinoma (AGS) cells at 1.25, 2.5 and 5 μM. Combined treatment of 1 and TRAIL sensitized AGS cells to TRAIL-induced apoptosis at the aforementioned concentrations. Copyright © 2010 John Wiley & Sons, Ltd.

Keywords: Sida acuta; Malvaceae; cryptolepine; TRAIL-induced apoptosis; AGS cell lines.

Supporting information may be found in the online version of this article (Supplementary Material)

INTRODUCTION

Tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL), a member of the TNF superfamily, has emerged as a promising anticancer agent, because of its ability to selectively kill tumor cells. The *in vivo* administration of TRAIL is proved to be safe, unlike the other members of the TNF superfamily. The relative absence of toxic side effects of this naturally occurring cytokine, in addition to its antitumor properties, has led to its preclinical evaluation. But, TRAIL-resistance is a major problem of its therapy, as a considerable number of cancer cells, especially some highly malignant tumors, are resistant to apoptosis induction by TRAIL (Ishibashi and Ohtsuki, 2008). A search for compounds capable of abrogating TRAIL resistance has, thus, become an important strategy for anticancer drug discovery. Different chemotherapeutic agents including a number of natural products are reported to have TRAIL-resistance overcoming activity. For example, luteolin, curcumin, resveratrol, indole-3-carbinol, flavopiridol and apigenin have received attention as anticancer agents. In the search for bioactive natural products from natural resources, a number of natural products with TRAIL-resistance overcoming activity were previously identified (Ishibashi and Ohtsuki, 2008).

Sida acuta Burm. (Malvaceae), locally known as 'Berela', is a shrub distributed in all areas of Bangladesh and other tropical countries. Its leaves are traditionally used as diuretic, demulscent, etc. The roots are used as tonic, diaphoretic and antipyretic (Kirtikar and Basu, 1975). It was reported as having antiplasmodial, antimicrobial, analgesic, free radical scavenging, and apoptosis inducing activities. Previously isolated constituents include alkaloids, tocopherols and triterpenoids, sterols, polyphenols and glycosides (Karou *et al*., 2007). Bioassay guided separation of the MeOH extract of whole plants of *Sida acuta* led to the isolation of compounds **1–3**. Here, the sensitizing effect of compound **1** on AGS cells to TRAIL-induced apoptosis is reported for the first time.

* Correspondence to: Professor Masami Ishibashi, Laboratory of Natural Products Chemistry, Graduate School of Pharmaceutical Sciences, Chiba University, 1-33Yayoi-cho, Inage-ku, Chiba 263-8522, Japan.
E-mail: mish@p.chiba-u.ac.jp

MATERIALS AND METHODS

Plant material. Whole plants of *S. acuta* were collected from Dhaka, Bangladesh in 2006 and taxonomically validated by the experts at Bangladesh National Herbarium. A voucher specimen was deposited at Khulna University, Bangladesh (R-009) for future reference.

Extraction and isolation. The MeOH extract (9.7 g, yield: 6.5%) of *S. acuta* was chromatographed over Diaion HP-20 (4.5 × 35 cm) to exclude the chlorophyll content. The chlorophyll-free fraction (8.1 g) was then suspended in 10% aq. MeOH and partitioned between hexane, EtOAc and BuOH. The EtOAc extract (1.9 g) was subjected to silica gel PSQ100B column chromatography (2.5 × 45 cm) using a $CHCl_3$–MeOH solvent system with increasing polarity to afford four fractions, 3A–3D. Fraction 3B (242 mg) was recrystallized to give compound **3** (40 mg). Fraction 3C (831 mg) was chromatographed over silica gel PSQ100B (2.5 × 45 cm) using a EtOAc–MeOH solvent system to give compound **1** (85 mg) and **2** (332 mg).

TRAIL-resistance overcoming activity. TRAIL-resistance overcoming activity of the extracts and isolates was assessed by comparison of cell growth inhibitory activity in the presence and absence of TRAIL against AGS cell lines, as reported previously (Ahmed *et al*., 2008).

Received 08 March 2010
Revised 13 April 2010
Accepted 14 April 2010

Hoechst staining. Apoptosis was detected by staining the treated cells with Hoechst 33342 reagent (Wako Pure Chemical Industries Ltd, Japan) as described previously (Adhami *et al*., 2007).

Caspase-3/7 activity. Caspase-3/7 activity was measured using a Caspase-Glo 3/7 assay kit (Promega) according to the manufacturer's instructions as described previously (Ohtsuki *et al*., 2009).

RESULTS AND DISCUSSION

In a screening programme for abrogating TRAIL-resistance, medicinal plants of Bangladesh were explored. Bioassay guided separation of MeOH extract of whole plants of *Sida acuta* led to the isolation of compounds **1**–**3**. The structure of **1** was elucidated as an alkaloid, known as cryptolepine (Yang *et al*., 1999) and compounds **2** and **3** were determined as kaempferol glycosides, known as kampferol-3-*O*-α-L-rhamnopyranosyl-β-D-glucopyranoside (Yoon *et al*., 2007) and kampferol-3-*O*-β-D-glucopyranoside (Okuyama *et al*., 1978), respectively (Fig. 1).

The isolated compounds (**1**–**3**) were evaluated for their activity in overcoming TRAIL resistance in AGS cells. Recently, this cell line has been used widely as a model system for evaluating cancer cell apoptosis, and is reported to be refractory to apoptosis induction by TRAIL (Srivastava, 2001). To assess the effects of **1**–**3**, TRAIL, or their combined treatment on cell viability, AGS cells were treated with the indicated agents and subjected to FMCA. As shown in Fig. 2, treatment with 100 ng/mL TRAIL for 24 h resulted in only a slight decrease in cell viability (91%). Luteolin (Horinaka *et al*., 2005), used as a positive control, produced about 50% more inhibition along with TRAIL than the agent alone at 17.5 μM. Combined treatment of AGS cells with 100 ng/mL TRAIL and 1.25 or 2.5 μM of **1** reduced the cell viability to 40% and 19% of control levels ($p < 0.01$), respectively, which was 32% and 50% more than the agent alone, suggesting a possible synergism between

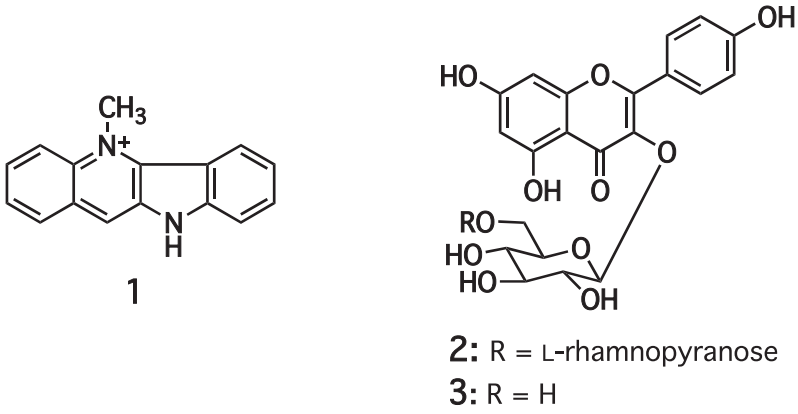

Figure 1. Structures of compounds **1**–**3**.

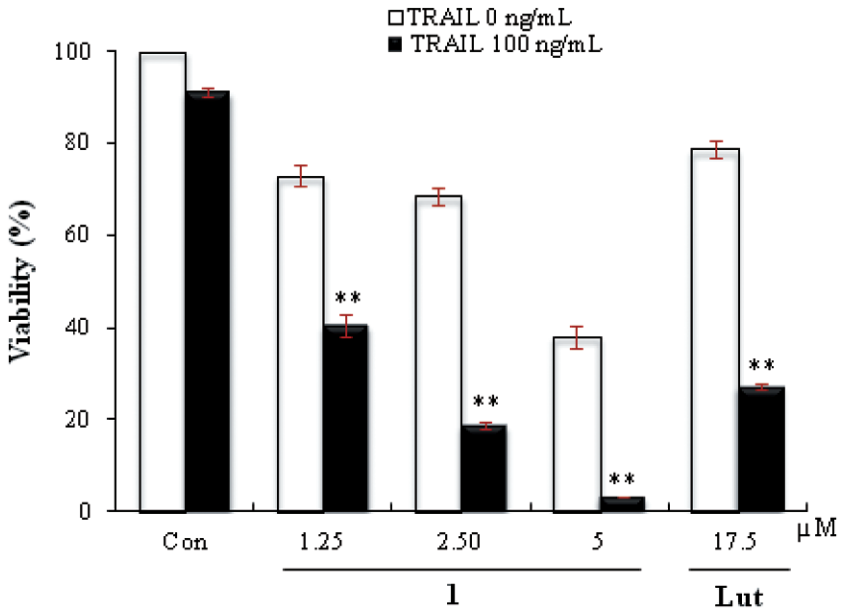

Figure 2. Effect of compound **1** on the viability of AGS cells. Effect of compound **1**, luteolin (positive control: Lut) and TRAIL treatment, alone and in combination, on the viability of AGS cells. Cells were seeded in a 96-well culture plate (6×10^3 cells per well) for 24 h and then treated with indicated concentrations (μM) of the compounds and/or TRAIL for 24 h. Cell viability was determined by fluorometric microculture cytotoxicity assay (FMCA). The bar represents the mean ($n = 3 \pm$ SD). Significance was determined by Student's *t*-test $p < 0.01$ (**) vs control (Con).

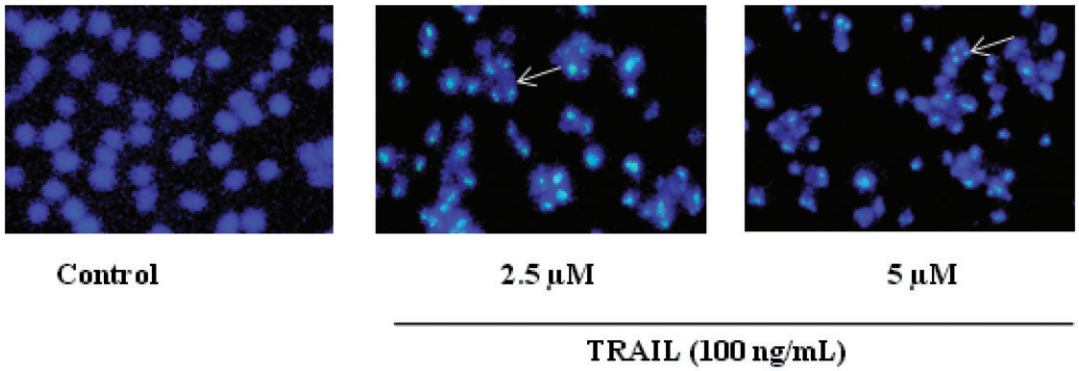

Figure 3. Effect of compound **1** on induction of apoptosis in AGS cells. Effect of **1** and TRAIL treatment, alone and in combination, on apoptosis of human gastric adenocarcinoma cells. The AGS cells were grown on cell culture dishes and treated as described in 'Experimental' and apoptosis was detected by Hoechst 33342 stain. Representative photomicrographs from each treatment group showing induction of apoptosis (bright fluorescence indicated by arrow).

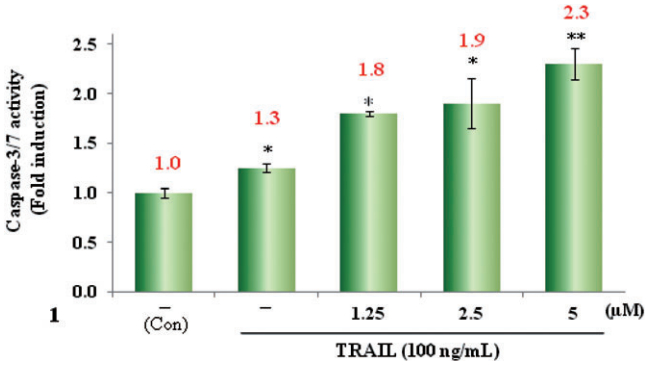

Figure 4. Effect of compound **1** on caspase-3/7 activity in AGS cells. Effect of combined treatment of **1** and TRAIL, on caspase-3/7 activity in human gastric adenocarcinoma cells. Values on top of the columns represent relative fold-induction compared with control considered as 1. Significance was determined by Student's *t*-test (** $p < 0.01$; * $p < 0.01$) vs control (Con).

the two agents. Compounds **2** and **3**, however, did not show TRAIL-resistance overcoming activity (data not shown). Cryptolepine, reported as a candidate antitumor agent (Laryea *et al.*, 2009), exhibits potent cytotoxic activity against a wide variety of cancer cells, including human leukemia HL-60 cells (Dassonneville *et al.*, 2000). It induced cell cycle arrest and apoptosis by activating mitochondrial release of cytochrome c. Here, for the first time, its TRAIL-resistance overcoming activity against AGS cells was found.

To ascertain whether the decrease in cell viability produced by compound **1** was caused by apoptotic cell death, the treated cells were stained after 24 h with Hoechst 33342 reagent. Apoptotic nuclei with condensed chromatins stained more brightly in the treated cells than the normal cells (Fig. 3), suggesting that the cell death was due to apoptosis (Llobet *et al.*, 2000).

The effect of **1** on caspase-3/7 activity in AGS cells was checked to ascertain whether the apoptosis induced by **1** was mediated through caspase activation. Caspase-3/7 are known as effector caspases, and after being activated by the initiator caspases (caspase-8/9), it induced apoptosis. It was observed that the treatment of AGS cells with **1** in combination with TRAIL (100 ng/mL), increased caspase-3/7 activity 1.8-, 1.9- and 2.3-fold, at 1.25, 2.5 and 5 μM, respectively, compared with the control after 12 h (Fig. 4). From these results it was confirmed that compound **1** induced apoptosis through caspase-3/7 activation (Ohtsuki *et al.*, 2009).

Although the molecular mechanisms responsible for the sensitizing effect of cryptolepine (**1**) are not described here in detail, it can be concluded that **1** sensitized AGS cells to TRAIL-induced apoptosis through caspase-3/7 activation.

Acknowledgement

This work was partly supported by the Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (JSPS).

Conflict of Interest

The authors have declared that there is no conflict of interest.

Supporting Information

Detailed protocols for TRAIL-resistance overcoming activity, Hoechst staining and caspase-3/7 activity along with the ^{1}H and ^{13}C NMR data for compound **1** are available as supporting information.

REFERENCES

Adhami VM, Malik A, Zaman N *et al.* 2007. Combined inhibitory effects of green tea polyphenols and selective cyclooxygenase-2 inhibitors on the growth of human prostate cancer cells both *in vitro* and *in vivo*. *Clin Cancer Res* **13**: 1611–1619.

Ahmed F, Ohtsuki T, Aida W, Ishibashi M. 2008. Tyrosine derivatives isolated from *Streptomyces* sp. IFM 10937 in a screening program for TRAIL-resistance-overcoming activity. *J Nat Prod* **71**: 1963–1966.

Dassonneville L, Lansiaux A, Wattelet A *et al.* 2000. Cytotoxicity and cell cycle effects of the plant alkaloids cryptolepine and neocryptolepine: relation to drug-induced apoptosis. *Eur J Pharmacol* **409**: 9–18.

Horinaka M, Yoshida T, Shiraishi T *et al.* 2005. Luteolin induces apoptosis via death receptor 5 up-regulation in human malignant tumor cells. *Oncogene* **24**: 7180–7189.

Ishibashi M, Ohtsuki T. 2008. Studies on search for bioactive natural products targeting TRAIL signaling leading to tumor cell apoptosis. *Med Res Rev* **28**: 688–714.

Karou D, Nadembega WMC, Ilboudo DP *et al.* 2007. *Sida acuta* Burm. f.: a medicinal plant with numerous potencies. *Afr J Biotechnol* **6**: 2953–2959.

Kirtikar KR, Basu BD. 1975. *Indian Medicinal Plants*, 2nd edn. Bishen Singh-Mahendrapal Singh: India.

Laryea D, Isaksson A, Wright CW, Larsson R, Nygren P. 2009. Characterization of the cytotoxic activity of the indoloquinoline alkaloid cryptolepine in human tumour cell lines and primary cultures of tumour cells from patients. *Invest New Drugs* **27**: 402–411.

Llobet D, Eritja N, Encinas M *et al*. 2000. CK2 controls TRAIL and Fas sensitivity by regulating FLIP levels in endometrial carcinoma cells. *Oncogene* **27**: 2513–2524.

Ohtsuki T, Kikuchi H, Koyano T, Kowithayakorn T, Sakai T, Ishibashi M. 2009. Death receptor 5 promoter-enhancing compounds isolated from *Catimbium speciosum* and their enhancement effect on TRAIL-induced apoptosis. *Bioorg Med Chem* **17**: 6748–6754.

Okuyama T, Hosoyama K, Kiraga Y, Kurono G, Takemoto T. 1978. The constituents of *Osmunda* spp. II. : A new flavonol glycoside of *Osmunda asiatica. Chem Pharm Bull* **26**: 3071–3074.

Srivastava RK. 2001. TRAIL/Apo-2L: Mechanisms and clinical applications in cancer. *Neoplasia* **3**: 535–546.

Yang S-W, Abdel-Kader M, Malone S *et al*. 1999. Synthesis and biological evaluation of analogues of cryptolepine, an alkaloid isolated from the Suriname Rainforest. *J Nat Prod* **62**: 976–983.

Yoon KD, Jeong DG, Hwang YH, Ryu JM, Kim J. 2007. Inhibitors of osteoclast differentiation from *Cephalotaxus koreana*. *J Nat Prod* **70**: 2029–2032.

