Journal of Natural Medicines (2018) 72:260–266
https://doi.org/10.1007/s11418-017-1147-9

ORIGINAL PAPER

Potent apoptosis-inducing activity of erypoegin K, an isoflavone isolated from *Erythrina poeppigiana*, against human leukemia HL-60 cells

Kiyomi Hikita[1] · Natsuki Hattori[1] · Aya Takeda[1] · Yuko Yamakage[1] · Rina Shibata[1] · Saori Yamada[1] · Kuniki Kato[2] · Tomiyasu Murata[1] · Hitoshi Tanaka[2] · Norio Kaneda[1]

Received: 9 June 2017 / Accepted: 26 October 2017 / Published online: 18 November 2017

Abstract
Erypoegin K is an isoflavone isolated from the stem bark of *Erythrina poeppigiana*. It contains a furan group at the A-ring of the core isoflavone structure and can inhibit the activity of glyoxalase I, an enzyme that catalyzes the detoxification of methylglyoxal (MG), a by-product of glycolysis. In the present study, we found that erypoegin K has a potent cytotoxic effect on human leukemia HL-60 cells. Its cytotoxic effect was much stronger than that of a known glyoxalase I inhibitor *S*-*p*-bromobenzylglutathione cyclopentyl diester. Conversely, erypoegin K demonstrated weak cytotoxicity toward normal human peripheral lymphocytes. The treatment of HL-60 cells with erypoegin K significantly induced caspase-3 activity, whereas the pretreatment of the cells with caspase-3 inhibitor suppressed erypoegin K-induced cell death. Furthermore, nuclear condensation and apoptotic genome DNA fragmentation were observed in erypoegin K-treated HL-60 cells. These results indicated that the observed cell death was mediated by apoptosis. In addition, the toxic compound MG was highly accumulated in the culture medium of erypoegin K-treated HL-60 cells, suggesting that cell apoptosis was triggered by extracellular MG. The present study showed that erypoegin K has a potent apoptosis-inducing effect on cancerous cell lines, such as HL-60.

Keywords *Erythrina poeppigiana* · Erypoegin K · Apoptosis · Glyoxalase I · Methylglyoxal · HL-60 cells

Introduction

Erythrina poeppigiana is distributed in South America, Africa, and Asia, and is planted as an ornamental plant in Okinawa Prefecture, Japan. The plant contains various types of isoflavonoids and alkaloids [1–4], some of which exhibit anti-viral activity, including inhibition of human immunodeficiency virus-1 replication [5], as well as anti-microbial [6, 7], anti-tumor [8], and estrogenic [9, 10] properties. In the current study, during the isolation of bioactive constituents from the stem bark of *E. poeppigiana* (Family: Leguminosae), we isolated erypoegin K, an isoflavone, for the first time and reported its inhibitory activity against recombinant human glyoxalase I (hGLO I) [11].

GLO I is an enzyme involved in the detoxification pathway of the toxic compound methylglyoxal (MG) produced as a by-product of glycolysis [12]. It catalyzes the reaction by which MG is converted into hemimercaptal using the reduced form of glutathione (GSH). Hemimercaptal is subsequently converted by glyoxalase II (GLO II) into the nontoxic product D-lactic acid [12, 13]. Tumor cells typically depend on glycolysis, rather than on the Krebs cycle, for ATP generation under both aerobic and anaerobic conditions. This phenomenon is referred to as the Warburg effect [14]. Multiple human cancer types, including breast [15], colon [16], lung [17], and liver [18, 19] cancers, as well as melanoma [20], express elevated levels of GLO I. The increase in GLO I expression is thought to mediate the survival of cancer cells under the condition of high MG levels. GLO I is also associated with multidrug resistance

✉ Norio Kaneda
nkaneda@meijo-u.ac.jp

[1] Laboratory of Analytical Neurobiology, Faculty of Pharmacy, Meijo University, Yagotoyama 150, Tempaku, Nagoya 468-8503, Japan

[2] Laboratory of Natural Product Chemistry, Faculty of Pharmacy, Meijo University, Yagotoyama 150, Tempaku, Nagoya 468-8503, Japan

in cancer cells [21, 22] and metastasis in certain types of carcinoma [23].

S-*p*-bromobenzylglutathione (BBG) is a known GLO I inhibitor, which is a bromobenzyl derivative of GSH. Because BBG is a water-soluble compound, a membrane-permeable BBG derivative, *S*-*p*-bromobenzylglutathione cyclopentyl diester (BBGC), is utilized for in vivo studies [17, 21, 24]. BBGC reportedly exhibits cytotoxicity and anti-tumor activity against several tumor cell lines and in tumor-bearing model animals [17, 24]. These findings suggest that selective GLO I inhibitors may function as effective tumor-specific anti-tumor agents [25]. In the present study, we investigated the effect of erypoegin K on the viability of cancerous HL-60 cells and compared it with that on human peripheral lymphocytes.

Results and discussion

Erypoegin K is an isoflavone compound (chemical structure illustrated in Fig. 1) containing a characteristic furan ring at the A-ring.

We examined the effects of erypoegin K on the viability of human acute promyelocytic leukemia HL-60 cells. The cells were treated with various concentrations of erypoegin K, and their viability was evaluated by MTS assay after incubation for 48 h. As depicted in Fig. 2, erypoegin K potently reduced the viability of HL-60 cells. The IC_{50} value was found to be 0.175 ± 0.004 μM, which was 20-fold more potent than that of a known GLO I inhibitor, BBGC (IC_{50} = 3.59 ± 0.10 μM).

We used human peripheral lymphocytes as a model to examine the effect of erypoegin K on normal (noncancerous) cells. As illustrated in Fig. 3, the cytotoxic effect of erypoegin K on normal cells was weak, suggesting that

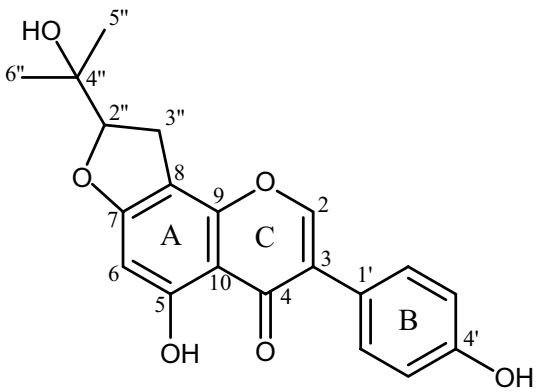

Fig. 1 Chemical structure of erypoegin K. The numbering and the A-, B-, and C-ring characteristics of isoflavones are shown

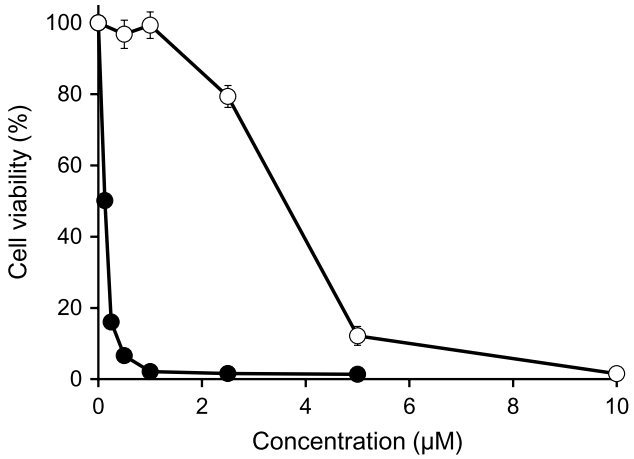

Fig. 2 Effects of erypoegin K on the viability of HL-60 cells. HL-60 cells (1.2×10^4 cells/well) were treated with various concentrations of erypoegin K for 48 h. The values are presented as mean ± SE of three independent experiments. Triplicate measurements were obtained in each experiment. Open circle, BBGC; closed circle, erypoegin K

the cytotoxic activity of erypoegin K might be specific to cancerous cell lines such as HL-60.

To determine whether erypoegin K-induced cell death occurred by apoptosis, we evaluated the enzymatic activity of caspase-3, a marker of apoptotic cell death. As depicted in Fig. 4, caspase-3 activity was strongly enhanced in HL-60 cells treated with 15 μM erypoegin K, and with 15 μM BBGC used as a positive control of

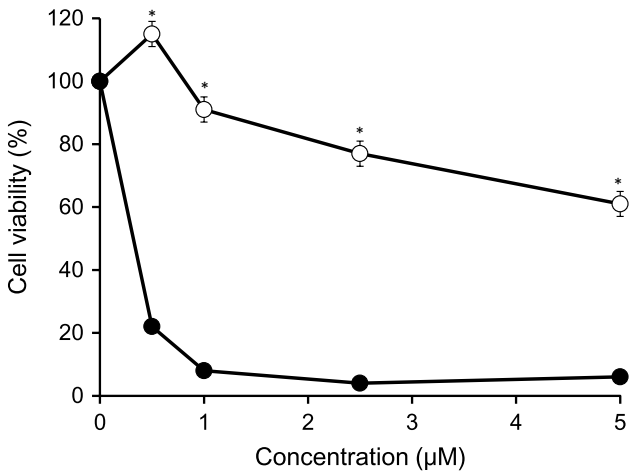

Fig. 3 Effect of erypoegin K on the viability of human peripheral lymphocytes. Human peripheral lymphocytes ($1.0–1.5 \times 10^5$ cells/well) and HL-60 cells (1.2×10^4 cells/well) were treated with various concentrations of erypoegin K for 48 h. The values are presented as mean ± SE of three independent experiments. Triplicate measurements were obtained in each experiment. The significance of differences was evaluated using Student's *t* test. *$p < 0.01$ vs. HL-60 cells. Open circle, human peripheral lymphocytes; closed circle, HL-60 cells

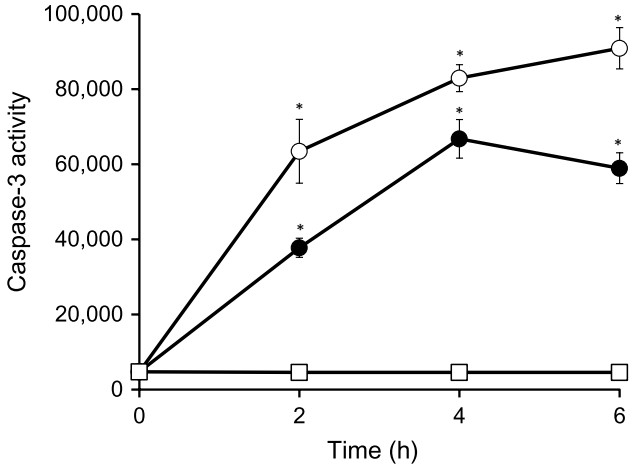

Fig. 4 Induction of caspase-3 activity in HL-60 cells treated with erypoegin K or BBGC. HL-60 cells (5.0×10^5 cells/well) were treated with 15 μM erypoegin K, 15 μM BBGC, or DMSO for the indicated time period. Caspase-3 activity was measured using a Caspase-3 Fluorometric Assay Kit, and the activity was expressed as an arbitrary unit of fluorescence intensity in proportion to the reaction product. The values are presented as mean ± SE of three independent experiments. Triplicate measurements were obtained in each experiment. The significance of differences was evaluated using Student's *t*-test. $*p < 0.01$ vs. control. Open circle, erypoegin K; closed circle, BBGC; open square, DMSO (control)

apoptosis-inducing agent in HL-60 cells [24]. To confirm this indication, we examined the effect of a caspase-3 inhibitor, Z-DEVD-FMK, on the viability of HL-60 cells treated with erypoegin K. As depicted in Fig. 5, the pretreatment of the cells for 2 h with the Z-DEVD-FMK rescued the viability in cells treated with erypoegin K for 6 h or 24 h, indicating the involvement of caspase-3 in the signaling pathway of cell death by erypoegin K. We further examined nuclear condensation and genome DNA fragmentation, both of which are characteristic features of apoptotic cells. The nuclear condensation was observed in cells treated with 10 μM erypoegin K for 6 h, whereas no condensation was observed in cells treated with DMSO (Fig. 6). A typical DNA fragmentation with a ladder-like pattern on agarose gel electrophoresis was visible in cells treated with 10 μM erypoegin K for 10 h (Fig. 7). These data clearly demonstrated that erypoegin K-induced cell death is mediated by apoptosis.

MG is a reactive dicarbonyl compound that is mainly formed as a metabolic by-product of glycolysis [24]. Reportedly, extracellular MG triggers the intracellular production of free radicals, which in turn easily interact with proteins, lipids, and DNA [26, 27]. These reactions may be closely associated with its cytotoxicity [28]. In fact, exogenously added MG was found to induce apoptosis in human leukemia HL-60 [29] and U937 [30] cells as well as in human hepatocyte HepG2 cells [31] and human osteoblasts [32]. Furthermore, BBGC has been shown to cause MG generation by HL-60 cells and to induce apoptosis [24]. We have previously demonstrated that erypoegin K can inhibit human recombinant GLO I enzyme activity [11]. This observation prompted us to measure MG levels in HL-60 cells treated with erypoegin K to determine whether erypoegin K can actually inhibit intracellular GLO I. Consequently, MG was not detected in the lysates of HL-60 cells treated with 10 μM erypoegin K (data not shown); however, high levels

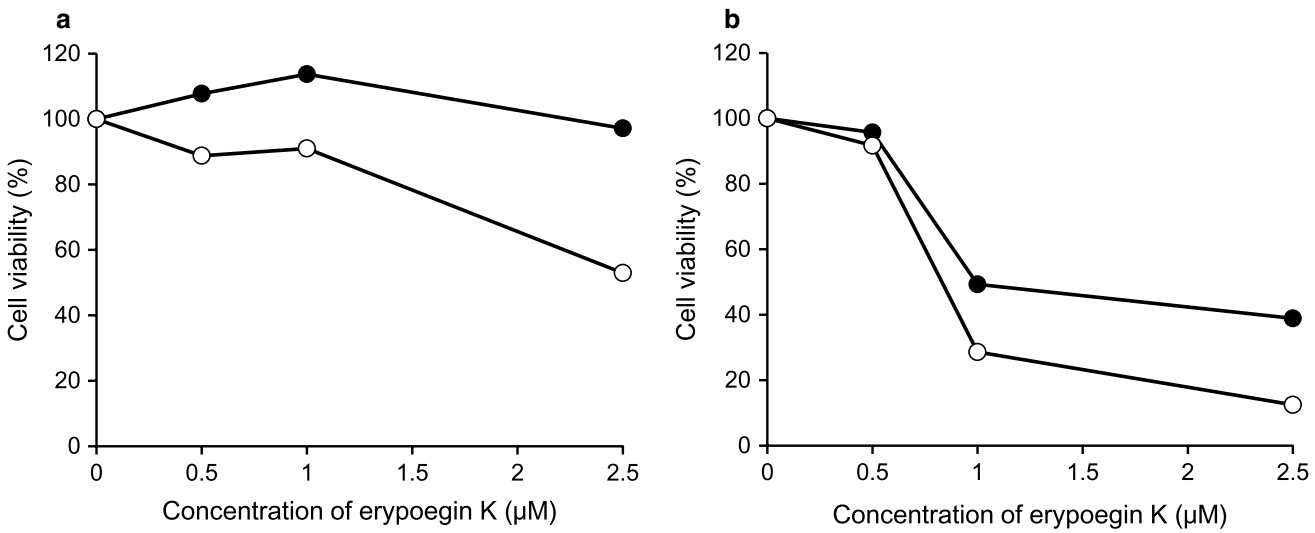

Fig. 5 Effect of the caspase-3 inhibitor on the viability of HL-60 cells treated with erypoegin K. HL-60 cells were treated with various concentrations of erypoegin K in the presence or absence of the caspase-3 inhibitor Z-DEVD-FMK for 6 h (**a**) or 24 h (**b**). The inhibitor was added 2 h prior to treatment with erypoegin K. Duplicate measurements were obtained in each experiment. Open circle, without caspase-3 inhibitor; closed circle, with caspase-3 inhibitor (100 μM)

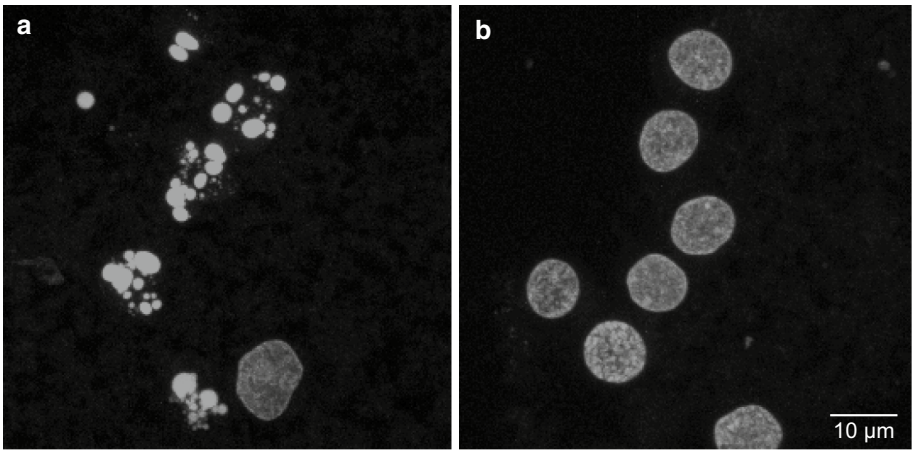

Fig. 6 Detection of DNA condensation. HL-60 cells (8.0×10^5 cells/well) were treated with 10 μM erypoegin K or DMSO for 6 h. The nuclei were stained with Hoechst 33342 and fixed with 5% paraformaldehyde. **a** erypoegin K; **b** DMSO (control)

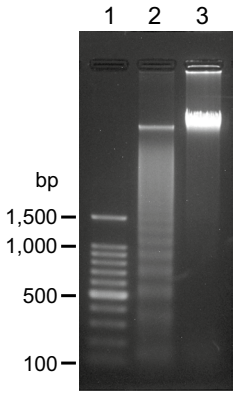

Fig. 7 Detection of apoptotic genome DNA ladder. HL-60 cells (2.0×10^5 cells/dish) were treated with 10 μM erypoegin K or DMSO for 10 h. Genomic DNA was extracted from the cells using Apoptosis Ladder Detection Kit Wako and separated by 1.5% agarose gel electrophoresis. Lane 1, 100-bp size marker; lane 2, erypoegin K; lane 3, DMSO (control)

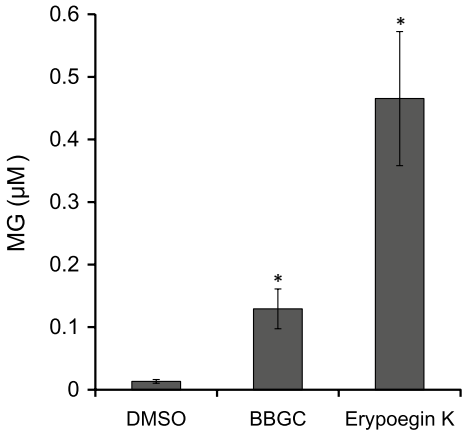

Fig. 8 MG concentration in the culture medium of HL-60 cells treated with erypoegin K or BBGC. HL-60 cells (1.5×10^6 cells/dish) were treated with 10 μM erypoegin K, 10 μM BBGC, or DMSO (control) for 24 h. MG concentration in the culture medium was measured using HPLC/UV. The values are presented as mean ± SE of three independent experiments. Duplicate measurements were obtained in each experiment. The significance of differences was evaluated using Student's *t* test. $*p < 0.05$ vs. control

of MG were detected in the culture medium after 24 h of the treatment; the levels were much higher than those in the medium obtained from HL-60 cells treated with 10 μM BBGC (Fig. 8). These data strongly suggested that erypoegin K inhibits intracellular GLO I activity, which leads to MG production and accumulation in the culture medium.

The cytotoxic effect of exogenously added MG was investigated at a sub-mM to mM level [29–32]. In our preliminary study, we found that the level of MG added in culture medium containing 10% fetal bovine serum (FBS, without cells) decreased rapidly, possibly due to covalent bond formation with proteins and/or amino acids present in the medium (data not shown). Therefore, there is a possibility that much higher levels of MG are released from the cells treated with erypoegin K than those shown in Fig. 8.

Erypoegin K that naturally occurs in *Erythrina* plants is a racemic mixture containing a chiral carbon at the C-2″ position (Fig. 1). Thus, our next aim is to elucidate whether there are any differences in the biological activities of the two optical isomers of erypoegin K, especially in their apoptosis-inducing activity against cancerous cell lines. These points are now under investigation.

In the present study, we demonstrated that erypoegin K, a compound isolated from *E. poeppigiana*, potently reduced the viability of and induced apoptosis in HL-60 cells through GLO I activity inhibition. The cytotoxic effect of erypoegin K was specific to cancerous HL-60 cells and not to human peripheral lymphocytes. These findings suggest that erypoegin K might provide the basis for a novel and selective cancer therapy.

Experimental

Preparation of erypoegin K

The methods used to prepare erypoegin K, including methanol extraction, silica gel chromatography, and reversed-phase HPLC, were conducted as previously described [11].

The purity of the compounds was evaluated using reversed-phase HPLC [11].

HL-60 cell cultures

Human acute promyelocytic leukemia HL-60 cells were obtained from RIKEN cell bank (Riken, Tokyo, Japan) and cultured in RPMI1640 medium supplemented with 10% FBS, 100 units/ml penicillin, and 100 µg/ml streptomycin at 37 °C in a humidified atmosphere of 5% CO_2 and 95% air.

Human peripheral lymphocyte cultures

Human peripheral lymphocytes from healthy volunteers were isolated using the Percoll–Hypaque density gradient method [33] with a Percoll isolation kit (Lymphoprep, Axis-Shield, Oslo, Norway), according to the manufacturer's instructions. The isolated lymphocytes were precultured for 24 h in RPMI1640 medium supplemented with 10% FBS, 100 units/ml penicillin, and 100 µg/ml streptomycin at 37 °C in a humidified atmosphere of 5% CO_2 and 95% air.

Cell viability assay

HL-60 cells in the log phase (approximately 1×10^6 cells/ml) were plated in a 96-well plate (Corning, NY, USA) at a density of 1.2×10^4 cells/well. Human peripheral lymphocytes were plated at a density of $1.0–1.5 \times 10^5$ cells/well. After 24 h of preincubation, the cells were treated with the test compound and cultured for an additional 48 h. Erypoegin K and BBGC were dissolved in DMSO (25 mM), and the final DMSO concentration in the cell culture medium was less than 0.4%. Cell viability was assessed using an MTS assay (CellTiter 96™ AQ_{ueous} One Solution Cell Proliferation Assay Kit, Promega, Madison, WI, USA) based on the production of water-soluble tetrazolium by mitochondrial dehydrogenase. In cells treated with the caspase-3 inhibitor Z-DEVD-FMK (R&D Systems Inc., Minneapolis, MN, USA), the inhibitor was added 2 h prior to the addition of the test compound.

Caspase assay

The enzymatic activity of caspase-3 in HL-60 cells was assessed using a Caspase-3 Fluorometric Assay Kit (R&D Systems Inc., Minneapolis, MN, USA), according to the manufacturer's instructions. HL-60 cells were plated in a 24-well plate (Corning, NY, USA) at a density of 5.0×10^5 cells/well. After 24 h, the cells were treated with the test compound for the indicated time period (h). Subsequently, the cells were washed thrice with phosphate buffered saline (PBS (−)) and were lysed using the lysis buffer supplied in the kit. Caspase-3 activity in the cell lysates was then measured using the Wallac 1420 ARVOsx microplate counter (excitation at 390 nm, emission at 510 nm; PerkinElmer, Waltham, MA, USA).

Detection of nuclear condensation

HL-60 cells were plated in a 24-well plate at a density of 8.0×10^5 cells/0.3 ml/well and treated with 10 µM erypoegin K for 6 h. The cells were collected into a 1.5-ml microtube by a brief centrifugation, with Hoechst 33342 reagent (Dojindo, Kumamoto, Japan) added (final concentration: 14.3 µg/ml), and stained for 30 min in a CO_2 incubator at 37 °C. Subsequently, the cells were fixed with the same volume of 10% paraformaldehyde in PBS (−) at room temperature for 30 min. Then, the cells were collected by a brief centrifugation and washed twice with PBS (−). Finally, cells were mounted with the VECTASHIELD™ medium (Vector laboratories, Burlingame, CA, USA), transferred to a slide glass, and observed under a fluorescence microscope (excitation at 352 nm, emission at 461 nm; Zeiss Axiophoto 2, Carl Zeiss, Jena, Germany).

DNA fragmentation assay

HL-60 cells were plated in a 3.5-cm dish (Corning, NY, USA) at a density of 2.0×10^5 cells/ml/dish and treated with 10 µM erypoegin K for 10 h. The cells were collected into 1.5-ml microtubes by a brief centrifugation. Genome DNA was extracted from the cell pellet using an Apoptosis Ladder Detection Kit Wako (Wako Pure Chemical Industries, Osaka, Japan), according to the manufacturer's instructions. The DNA fragments were separated by 1.5% agarose gel electrophoresis, stained with SYBR™ Green I, and observed with an UV transilluminator.

MG assay

MG levels in the culture medium and the cell lysate were determined as previously described [34] with some modifications. HL-60 cells were plated in a 6-cm dish (Corning, NY, USA) at a density of 1.5×10^6 cells/dish for 24 h and cultured in the presence of the test compound for the indicated time period (h). Subsequently, the cells were centrifuged ($153 \times g$ for 6 min), and the culture medium was collected and deproteinized using perchloric acid (final concentration: 0.5 M). The cells were washed thrice with PBS (−), homogenized by sonication (ultrasonic homogenizer VP-050, TAITEC, Saitama, Japan), and treated with perchloric acid (final concentration: 0.5 M). The samples were purified using a Sep-Pak Light tC18 cartridge (Waters, Milford, MA, USA). The flow-through fraction was collected, and MG was derivatized into 2-methylquinoxaline (2-MQ) by *o*-phenylenediamine for 4 h at room temperature.

Following the addition of 5-methylquinoxaline (5-MQ) as the internal standard, the reaction solution was applied to the Sep-Pak Light tC18 cartridge. Bound 2-MQ and 5-MQ were eluted from the cartridge using acetonitrile, and the eluent was concentrated to approximately 100 µl using a Speed-Vac SPD1010 system (Thermo Fisher Scientific, Waltham, MA, USA). 2-MQ and 5-MQ levels were measured using an HPLC platform (Jasco, Hachioji, Japan) equipped with an UV/VIS detector (UV-1570, wavelength: 315 nm; Jasco). The reversed-phase Develosil C30-UG-5 column (4.6 × 150 mm, 5 µm; Nomura Chemical, Aichi, Japan) was used, and the mobile phase comprised 10 mM KH_2PO_4 (pH 2.5; 80%) and acetonitrile (20%).

Statistical analysis

The significance of differences was estimated using Student's *t* test with Microsoft Excel (Excel 2013). $p < 0.05$ was considered to be significant.

Acknowledgements This work was partly supported by a Grant-in-Aid for Scientific Research (C) (JP16K08311) from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

Compliance with ethical standards

Conflict of interest The authors declare no conflicts of interest.

References

1. Tanaka H, Etoh H, Shimizu H, Oh-Uchi T, Terada Y, Tateishi Y (2001) Erythrinan alkaloids and isoflavonoids from *Erythrina poeppigiana*. Planta Med 67:871–873
2. Tanaka H, Oh-Uchi T, Etoh H, Shimizu H, Tateishi Y (2002) Isoflavonoids from the roots of *Erythrina poeppigiana*. Phytochemistry 60:789–794
3. Tanaka H, Oh-Uchi T, Etoh H, Sako M, Sato M, Fukai T, Tateishi Y (2003) An arylbenzofuran and four isoflavonoids from the roots of *Erythrina poeppigiana*. Phytochemistry 63:597–602
4. Tanaka H, Etoh H, Kulkarni A, Fukai T (2004) Phenolic constituents and alkaloids from *Erythrina* species. Recent Res Devel Phytochem 8:83–116
5. Tanaka H, Oh-Uchi T, Nitanda T, Baba M, Etoh H, Shimizu H (2002) Prenylated cinnamylphenols, HIV-1 replication inhibitors, from *Erythrina poeppigiana*. Ite Lett Batter New Technol Med 3:612–615
6. Sato M, Tanaka H, Yamaguchi R, Oh-Uchi T, Etoh H (2003) *Erythrina poeppigiana*-derived phytochemical exhibiting antimicrobial activity against *Candida albicans* and methicillin-resistant *Staphylococcus aureus*. Lett Appl Microbiol 37:81–85
7. Sato M, Tanaka H, Tani N, Nagayama M, Yamaguchi R (2006) Different antibacterial actions of isoflavones isolated from *Erythrina poeppigiana* against methicillin-resistant *Staphylococcus aureus*. Lett Appl Microbiol 43:243–248
8. Hikita K, Yamada S, Shibata R, Katoh M, Murata T, Kato K, Tanaka H, Kaneda N (2015) Inhibitory Effect of isoflavones from *Erythrina poeppigiana* on the growth of HL-60 human leukemia cells through inhibition of glyoxalase I. Nat Prod Commun 10:1581–1584
9. Djiogue S, Halabalaki M, Alexi X, Njamen D, Fomum ZT, Alexis MN, Skaltsounis A-L (2009) Isoflavonoids from *Erythrina poeppigiana*: evaluation of their binding affinity for the estrogen receptor. J Nat Prod 72:1603–1607
10. Djiogue S, Halabalaki M, Njamen D, Kretzschmar G, Lambrinidis G, Hoepping J, Raffaelli FM, Mikros E, Skaltsounis A-L, Vollmer G (2014) Erythroidine alkaloids: a novel class of phytoestrogens. Planta Med 80:861–869
11. Hikita K, Tanaka H, Murata T, Kato K, Hirata M, Sakai T, Kaneda N (2014) Phenolic constituents from stem bark of *Erythrina poeppigiana* and their inhibitory activity on human glyoxalase I. J Nat Med 68:636–642
12. Thornalley PJ (1990) The glyoxalase system: new developments towards functional characterization of a metabolic pathway fundamental to biological life. Biochem J 269:1–11
13. Racker E (1951) The mechanism of action of glyoxalase. J Biol Chem 190:685–696
14. Warburg O (1956) On respiratory impairment in cancer cells. Science 124:269–270
15. Rulli A, Carli L, Romani R, Baroni T, Giovannini E, Rosi G, Talesa V (2001) Expression of glyoxalase I and II in normal and breast cancer tissues. Breast Cancer Res Treat 66:67–72
16. Ranganathan S, Tew KD (1993) Analysis of glyoxalase-I from normal and tumor tissue from human colon. Biochim Biophys Acta 1182:311–316
17. Sakamoto H, Mashima T, Sato S, Hashimoto Y, Yamori T, Tsuruo T (2001) Selective activation of apoptosis program by *S-p*-bromobenzylglutathione cyclopentyl diester in glyoxalase I-overexpressing human lung cancer cells. Clin Cancer Res 7:2513–2518
18. Hu X, Yang X, He Q, Chen Q, Yu L (2014) Glyoxalase 1 is upregulated in hepatocellular carcinoma and is essential for HCC cell proliferation. Biotechnol Lett 36:257–263
19. Zhang S, Liang X, Zheng X, Huang H, Chen X, Wu K, Wang B, Ma S (2014) Glo1 genetic amplification as a potential therapeutic target in hepatocellular carcinoma. Int J Clin Exp Pathol 7:2079–2090
20. Bair WB 3rd, Cabello CM, Uchida K, Bause AS, Wondrak GT (2010) GLO1 overexpression in human malignant melanoma. Melanoma Res 20:85–96
21. Sakamoto H, Mashima T, Kizaki A, Dan S, Hashimoto Y, Naito M, Tsuruo T (2000) Glyoxalase I is involved in resistance of human leukemia cells to antitumor agent-induced apoptosis. Blood 95:3214–3218
22. Thornalley PJ, Rabbani N (2011) Glyoxalase in tumourigenesis and multidrug resistance. Semin Cell Dev Biol 22:318–325
23. Tanaka T, Kuramitsu Y, Wang Y, Baron B, Kitagawa T, Tokuda K, Hirakawa K, Yashiro M, Naito S, Nakamura K (2013) Glyoxalase 1 as a candidate for indicating the metastatic potential of SN12C human renal cell carcinoma cell clones. Oncol Rep 30:2365–2370
24. Thornalley PJ, Edwards LG, Kang Y, Wyatt C, Davies N, Ladan MJ, Double (1996) Antitumour activity of *S-p*-bromobenzylglutathione cyclopentyl diester *in vitro* and *in vivo*. Inhibition of glyoxalase I and induction of apoptosis. Biochem Pharmacol 51:1365–1372
25. Vince R, Wadd WB (1969) Glyoxalase inhibitors as potential anticancer agents. Biochem Biophys Res Commun 35:593–598
26. Desai KM, Wu L (2008) Free radical generation by methylglyoxal in tissues. Drug Metabol Drug Interact 23:151–173
27. Kalapos MP (2008) The tandem of free radicals and methylglyoxal. Chem Biol Interact 171:251–271
28. Talukdar D (2008) A brief critical overview of the biological effects of methylglyoxal and further evaluation of a methylglyoxal-based anticancer formulation in treating cancer patients. Drug Metabol Drug Interact 23:175–210

29. Kang Y, Edwards LG, Thornalley PJ (1996) Effect of methylglyoxal on human leukaemia 60 cell growth: modification of DNA G1 growth arrest and induction of apoptosis. Leuk Res 20:397–405
30. Okado A, Kawasaki Y, Hasuike Y, Takahashi M, Teshima T, Fujii J, Taniguchi N (1996) Induction of apoptotic cell death by methylglyoxal and 3-deoxyglucosone in macrophage-derived cell lines. Biochem Biophys Res Commun 225:219–224
31. Seo K, Ki SH, Shin SM (2014) Methylglyoxal induces mitochondrial dysfunction and cell death in liver. Toxicol Res 30:193–198
32. Chan WH, Wu HJ, Shiao NH (2007) Apoptotic signaling in methylglyoxal-treated human osteoblasts involves oxidative stress, c-Jun N-terminal kinase, caspase-3, and p21-activated kinase 2. J Cell Biochem 100:1056–1069
33. Boyum A (1968) Isolation of mononuclear cells and granulocytes from human blood. Scand J Clin Lab Invest Suppl 97:77–89
34. Chaplen FWR, Fahl WE, Cameron DC (1996) Method for determination of free intracellular and extracellular methylglyoxal in animal cells grown in culture. Anal Biochem 238:171–178